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GENESIS OF THE GANGRENOUS AND REPARATIVE PROCESSES IN TRENCH FOOT

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CHICAGO

THIS REPORT is part of a study of 19 cases of trench foot seen at Camp Butner General Hospital in the early months of 1945. Biopsies were done in 15 of the cases, and the results were presented in another report.[1] It was impossible to differentiate by any morphologic criteria between the tissues from the 15 patients with trench foot and those from 8 normal healthy controls. Except for the history of having been exposed to cold wet weather, there was no objective means of separating the men without any loss of tissue from other hospital patients with no history of cold injury.[2] Ten of the 19 men suffered injury severe enough to have lost some tissue, the loss varying from a part of a single toe to both feet. This report deals specifically with the pathologic aspects of these amputated specimens.

CLINICAL HISTORY

The clinical history of trench foot and related diseases has been amply presented in the recent and even in the ancient literature.[3] The

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1. Block, M.: Arch. Path. **44**:360, 1947.

2. (*a*) Krause, L.: Personal communication to the author. (*b*) Silverman, J.: Ann. Int. Med. **22**:702, 1946. (*c*) Block.[1]

3. (*a*) Larrey, D. J.: Mémoires de chirurgie militaire et campagnes, Paris, C. S. Smith, 1812. (*b*) Ungley, C. C., and Blackwood, W.: Lancet **2**:447, 1942. (*c*) Lesser, A.: Ann. Surg. **21**:257, 1945. (*d*) Patterson, R. H., and Anderson, F. M.: Surg., Gynec. & Obst. **80**:1, 1945. (*e*) Friedman, N.: Am. J. Path. **21**:387, 1945; (*f*) Am. J. Clin. Path. **16**:634, 1946. (*g*) Webster, D. R.; Woolhouse, F. M., and Johnston, J. L.: J. Bone & Joint Surg. **24**:185, 1942. (*h*) White, J. C.: New England J. Med. **228**:211 and (*i*) 241, 1943. (*j*) White, J. C., and Scoville, W.: ibid. **232**:415, 1944. (*k*) White, J. C., and Warren, S.: War Med. **5**:613, 1944. (*l*) Wieting: Zentralbl. f. Chir. **40**:593, 1913. (*m*) Wright, I. S., and Allen, E. V.: Bull. U. S. Army M. Dept., 1943, no. 65, p. 136. (*n*) Leriche, R., and Kunlin, J.: Progrès méd. **68**:167, 1940.

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first accurate account was written by Larrey[3a] on the basis of what he experienced during Napoleon's retreat from Moscow. In modern times, Wieting[31] gave a clear description of the disease as it occurred in the Balkan Wars.

In the present report the typical case was that of an infantryman of the 19 to 25 year age group, in previous good health, with the rank of private or corporal, who participated in the campaign of the American Armies in France and Germany in the winter of 1944-1945. His history is that of a patient who underwent a severe enough exposure to have suffered marked loss of tissue.

He arrived in France on Dec. 15, 1944 as a member of the 70th Infantry Division. He went into combat on January 2, 1945, and from that time on, his feet were continually cold and wet. The weather alternated between snow and rain, with days during which the ground was frozen solid for a few hours. The patient was wearing well fitting combat shoes over socks that were half wool and half cotton. He was usually able to alternate his socks during the first few days, drying his reserve pair against his body. Because his shoes were wet, his socks became wet within a few moments of changing. He never wore "shoepacs," because other men said they caused the feet to sweat profusely. He did not wear galoshes. During the last three days of combat he was unable to take time to remove his shoes.

About January 5, he noted that his feet were pale, swollen, painful, and gave a sensation of burning, and that numbness was developing. The next day he was able to get into his shoes only with great difficulty.

His company attacked on January 6, and he walked through the melting snow for twenty-four hours. On January 7 he was on outpost duty, lying in his foxhole, with his feet hanging down in the melted snow. The next morning his feet were blue and cold and much more painful. They burned and tingled, and he felt as though he were walking on stumps. By January 9, he was hardly able to walk, and when he turned in at the battalion aide station, his shoes had to be cut off because of the swelling of his feet.

The same day he was evacuated from his company, and on January 10, while he was on the way to a general hospital, blue-red, fluid-filled blisters began to appear on his toes. Beginning with January 9, his feet were kept at room temperature and thereafter became much more inflamed and painful. On January 12 gangrene developed in his toes, appearing first in the big toe of each foot as a black area under the nail and then affecting all the other toes. By January 16, the gangrenous skin reached the midtarsal areas, and the heels were covered with heavy dark hyperkeratotic skin, with some fairly normal tissue between toe and heel. The front halves of his feet were black and dried out like old shoe leather.

The black skin began to exfoliate about the end of January, leaving the skin on his heels a delicate pink, as after a mild burn. The peeling ceased at the ends of the metatarsal bones and a demarcation zone became evident, while the toes became even more dried out and contractures began at the phalangeal joints. By March he was able to walk, regardless of the gangrenous toes, which were but slightly disabling in spite of the extensiveness and severity of the gangrene.

He had been given injections of both penicillin and tetanus antitoxin. His feet were kept uncovered because when covered by blankets they were painful. On April 17 guillotine amputation was done bilaterally through the distal ends of the meta-

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tarsal bones, and on June 6 dermatome skin grafts were applied to the granulating surface of his feet.

The site of amputation broke down about three months later when his feet were trampled. In November of 1946 his feet and hands perspired profusely, the distal ends of his feet were blue-red, the skin was atrophic, the deeper tissue was hard and fibrous, and when his feet were hanging they became abnormally cyanotic and edematous. The dorsalis pedis and posterior tibial pulses were palpable. He could not keep his feet under blankets at night, and they were susceptible to extremes of weather.

This history was duplicated in thousands of other cases, the only variable being the intensity of exposure as it influenced the resultant injury. On the other hand, there was always a variation in the extent of injury in men exposed to the same environment. There can be little doubt that there was a marked individual difference in the quantitative reaction to cold. Some men had less severe injury, and within a few weeks after evacuation the gangrenous skin exfoliated, leaving a healthy pink covering on their feet. In others, with more severe injury, the whole of both feet were lost. Breakdown of tissue rarely occurred after amputation. Usually the feet gradually assumed a much more normal appearance, and the men were eventually able to keep their feet under blankets.

MATERIALS AND METHODS

The tissues examined were all obtained at amputation prior to the onset of any secondary infection. A demarcation zone had set in, and the mummified tissue had already begun to contract like an old paper bag.

All the tissues were fixed for four to six hours in formaldehyde-Zenker solution,[4] embedded in pyroxylin (nitrocellulose) and cut serially at 8 to 12 microns. At first all the tissues were stained with hematoxylin-eosin-azure II, Mallory-azocarmine,[5] orcein and phosphotungstic acid-hematoxylin. The phosphotungstic acid-hematoxylin staining was discontinued since it did not demonstrate elastic fibers as well as did staining with orcein, collagen fibers as well as did Mallory azocarmine staining or reticular fibers as well as did silver impregnation. Many of the tissues were also impregnated with silver by a modified Bielschowsky technic, and some of the latter were counterstained by the Mallory-azocarmine technic.

GROSS PATHOLOGIC FEATURES OF THE AMPUTATED SPECIMENS

Since little can be added to the careful descriptions recorded by Larrey,[3a] Friedman[3e] and Lesser,[3c] there is no necessity to enter into any detailed analysis of the results of the pathologic examination of the gross specimens. Regardless of how much tissue was lost, each of the amputation specimens was made up of a mummified gangrenous zone and a reactive or regenerative zone, usually separated by a demarcation zone.

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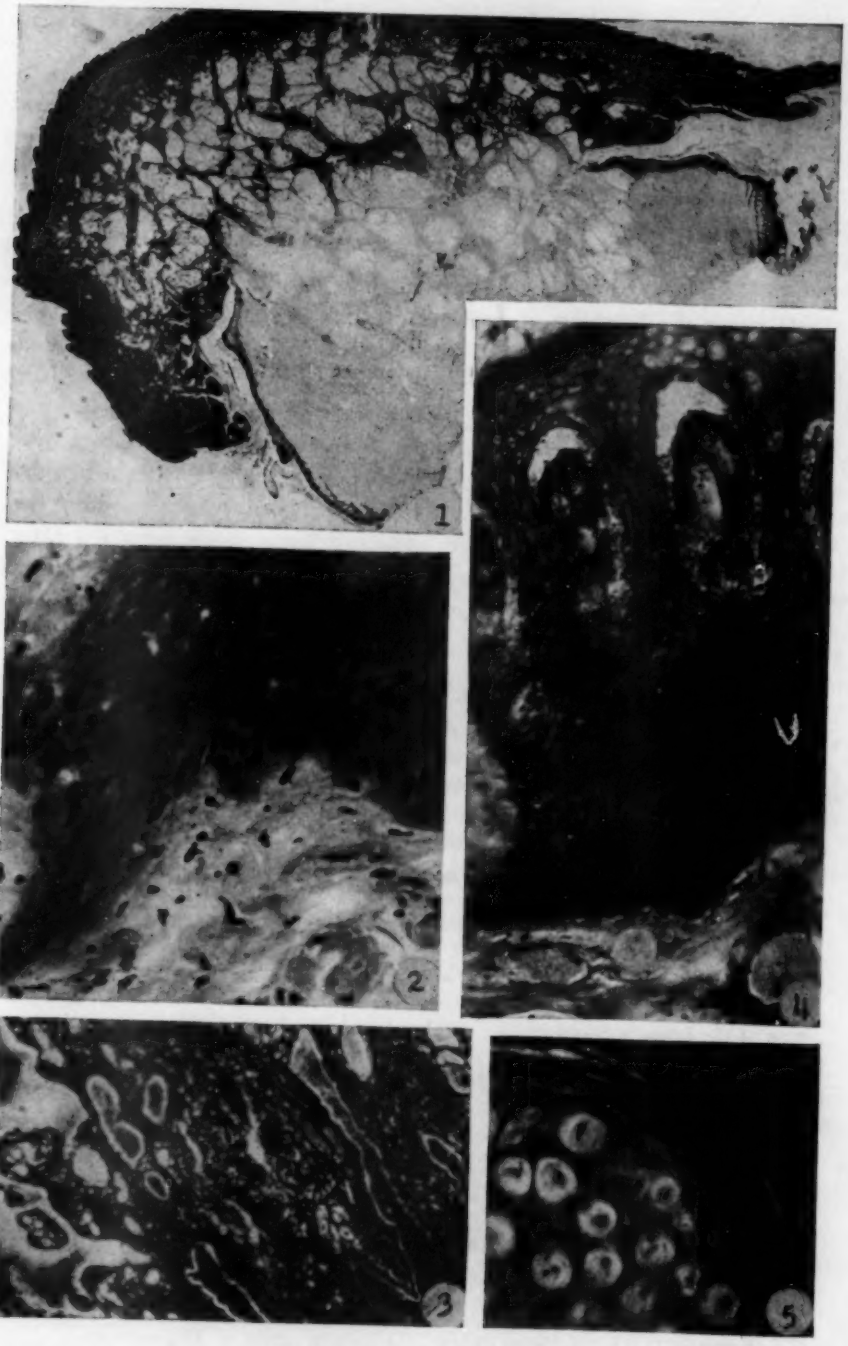
4. This is Zenker's solution to which, instead of acetic acid, solution of formaldehyde U.S.P. has been added to a concentration of 10 per cent.

5. This is Mallory's connective tissue stain with azocarmine G or B substituted for acid fuchsin.



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In the first zone the skin was horny and black and extended a variable distance into the toe or the foot; beneath it was found an odorless, slightly moist tissue. When the bone protruded, it was hard and dry. If the bone was buried in gangrenous tissue, it usually had a purple discoloration and a wet appearance, most marked at the epiphyses. Occasionally, when the black, gangrenous tissue was only a few millimeters thick, the tissue in the center of the toe was grossly normal.

The demarcation zone was rather softer and moister than the other two zones and made an indentation in the surface of the skin. It was odorless in the great majority of cases, since the amputations were performed before frank secondary infection had begun.

The third zone, which was only a few centimeters in length, was usually slightly erythematous and just slightly edematous. Thereafter, erythema and edema disappeared. The unamputated portions greatly resembled the feet of a patient who had not lost tissue, and, in general, those who had had amputations suffered from the same clinical symptoms as did those who had lost no tissue. Surprisingly, the severity of these symptoms was greatest in the group without loss of tissue.[6]

HISTOLOGIC DESCRIPTION

GANGRENOUS ZONE

Although the general histologic features, especially the fiber structure, were preserved, the tissues of the gangrenous zone had become desiccated and had lost their normal ability to be stained differently (figs. 1 and 3). The dead nuclei, but not the cytoplasm, were clearly seen (fig. 2). In the less severely damaged areas, the dead cells and fibers were well preserved, and, except for some hyalinization of the tissue, there was little distortion (figs. 3 and 4).

Epidermis.—In the more severely mummified areas the epidermis was a hyalinized structureless mass without even the normal amount of intercellular and intracellular fluid. When nuclei were still present, they were found as pyknotic masses in the stratum germinativum, especially near the sweat ducts (fig. 2). In the less severely mummified areas there were intercellular and extracellular vacuoles (fig. 4). No leukocytic infiltration or signs of regeneration were seen. The basement membrane was well preserved (fig. 3).

Sweat Glands.—The nuclei of the cells forming the sweat glands were pyknotic but still stainable. In silver impregnations the hyalinized cytoplasm and the surrounding hyalinized connective tissue were seen to be separated from each

6. Block.[1] Krause.[2a]

Fig. 1.—Low power view of an amputated toe illustrating the three zones: gangrene, demarcation and reaction. The viable epidermis is undercutting the gangrenous tissue. (Hematoxylin-eosin-azure II; × 6; Army Institute of Pathology negative 98470.)

Fig. 2.—Gangrenous area with dead, pyknotic nuclei clearly visible. (Hematoxylin-eosin-azure II; × 400; Army Institute of Pathology negative 98483.)

Fig. 3.—Essentially normal reticular fiber basement membrane and normal reticular fiber scaffolding. The fibers are collapsed on one another because of the desiccation. (Silver; × 115; Army Institute of Pathology negative 98474.)

Fig. 4.—Epidermal intracellular vacuolation and intercellular fluid in gangrenous but not severely mummified tissue. The vessels are dilated and obstructed by red cells. (Hematoxylin-eosin-azure II; × 185; Army Institute of Pathology negative 98481.)

Fig. 5.—Sclerotic but not mummified nerve. Collagen stains with aniline blue. (Mallory-azocarmine; × 450; Army Institute of Pathology negative 98485.)

other by a basement membrane (fig. 3, upper right). In a few cases the sweat glands underwent granular degeneration, most marked in the secretory portions. This degenerative process began as an accumulation of azocarminophilic granules similar to secretory granules in the apical ends of the cells. Then the nuclei degenerated, and the granules filled the cells until finally the gland was transformed into a mass of granules, still separated by an argyrophil basement membrane from the adjoining hyalinized connective tissue (fig. 3).

Nerves.—Many of the nerves were sclerosed (fig. 5); in some cases this was preceded, in others followed, by mummification. The sclerosis consisted of an increase in the endoneurial collagen. The onset of mummification was evidenced by a change in the staining by the Mallory-azocarmine technic. In partially mummified areas the collagen was stained, part by the aniline blue and part by the azocarmine (fig. 6*b*). In most severely mummified areas the whole nerve stained diffusely with azocarmine, but by means of silver impregnation one could demonstrate a fairly normal reticular fiber network, indicating that the reticular fiber scaffolding was still largely intact.

Dermis.—Papillary Layer: The papillary folds were usually wide, but this depended on the extent of dilatation of the vessels. With the Mallory-azocarmine technic, this layer showed a diffuse red stain, indicating mummification. Characteristically, there was rarely evidence of inflammation (figs. 1 to 4). The nuclei were pyknotic and elongated (fig. 2) and had undergone degeneration or decreased in number.

The most spectacular manifestation of injury was a massive dilatation of most of the blood vessels, so that it was impossible to differentiate arterioles from venules, because of compression of the walls. The lumens were filled with packed masses of erythrocytes, many of which were hemolyzed. There was no attempt at reorganization of these occluded vessels.

The reticular fibers were preserved, and perhaps slightly thickened (fig. 3). The collagenous fibers were hyalinized, usually mummified, and compressed on one another (fig. 2). The elastic fibers were rather variable in thickness and distribution.

Reticular Layer: In general, the changes seen here were the same as those described in the papillary layer. Owing to the presence of numerous venous sinusoids, the vascular dilatation was more spectacular here than in the papillary layer (figs. 3 and 4). However, even in these vessels a compressed but fairly normal reticular fiber network was found, although the collagenous fibers failed to stain normally with the Mallory-azocarmine technic. The mummification never proceeded as a wave advancing from superficial to deep tissues, but always began as patchy, focal areas. The elastic fibers were variable—some thick, others thin.

Glomuses were not seen. In spite of a careful review of the literature, only one reference to the appearance of the glomus in cold injury was noted.[7]

Subcutaneous Tissues.—Fat: The normal structure of the subcutaneous tissue was preserved (fig. 1). There was sometimes thickening of the collagenous fibers and reticular fibers around the fat lobules and fat cells. This intercellular tissue was often mummified. The process of sclerosis and mummification was most severe between the fat lobules and at the periphery of the lobule (fig. 8). Except near the zone of demarcation, there was no inflammation. Had it not been for occasional absence of nuclei, the dead fat cells would have looked almost normal.

Subcutaneous Vessels: The neurovascular septums were thickened and mummified. The vessels were greatly distended and filled with masses of red blood

7. Theis, F.: Arch. Phys. Therapy **21**:663, 1940.

cells. The mummification involved the smooth muscle cells first and to the greatest degree. In the most advanced cases the walls of the vessels were fused into a homogeneous mass with a few degenerated nuclei. In these cases the orcein-stained slides or the silver-impregnated slides often were useful in differentiating arteries from veins, since the elastic and reticular fibers were usually less severely involved than the other tissue components. (fig. 7).

Striated Muscle.—The mummification involved the muscle fibers more than it did the intermuscular connective tissue, which was increased in amount. The reticular fibers were well preserved. The most noticeable changes were a progressive but patchy hyalinization and a decrease in number of nuclei of the muscle cells.

Bone.—The bone spicules had undergone aseptic necrosis. In covered bones the marrow was a mass of granular debris which still suggested the basis structure of marrow, but in bones that protruded through the flesh the marrow had disappeared completely.

DEMARCATION ZONE

This zone, which consisted of a mass of degenerated leukocytes, divided the other two zones (fig. 1). Here the fiber scaffolding of the tissue had been destroyed. If this zone was diffuse and without intense leukocytic infiltration, the destruction of the fibers was incomplete (fig. 8). The demarcation zone occurred at any angle to the surface skin. It was no respecter of tissue layers and even cut through individual vessels (fig. 8). It was more closely adherent to the gangrenous zone than to the reactive zone, since it tended to split from the latter in sectioning.

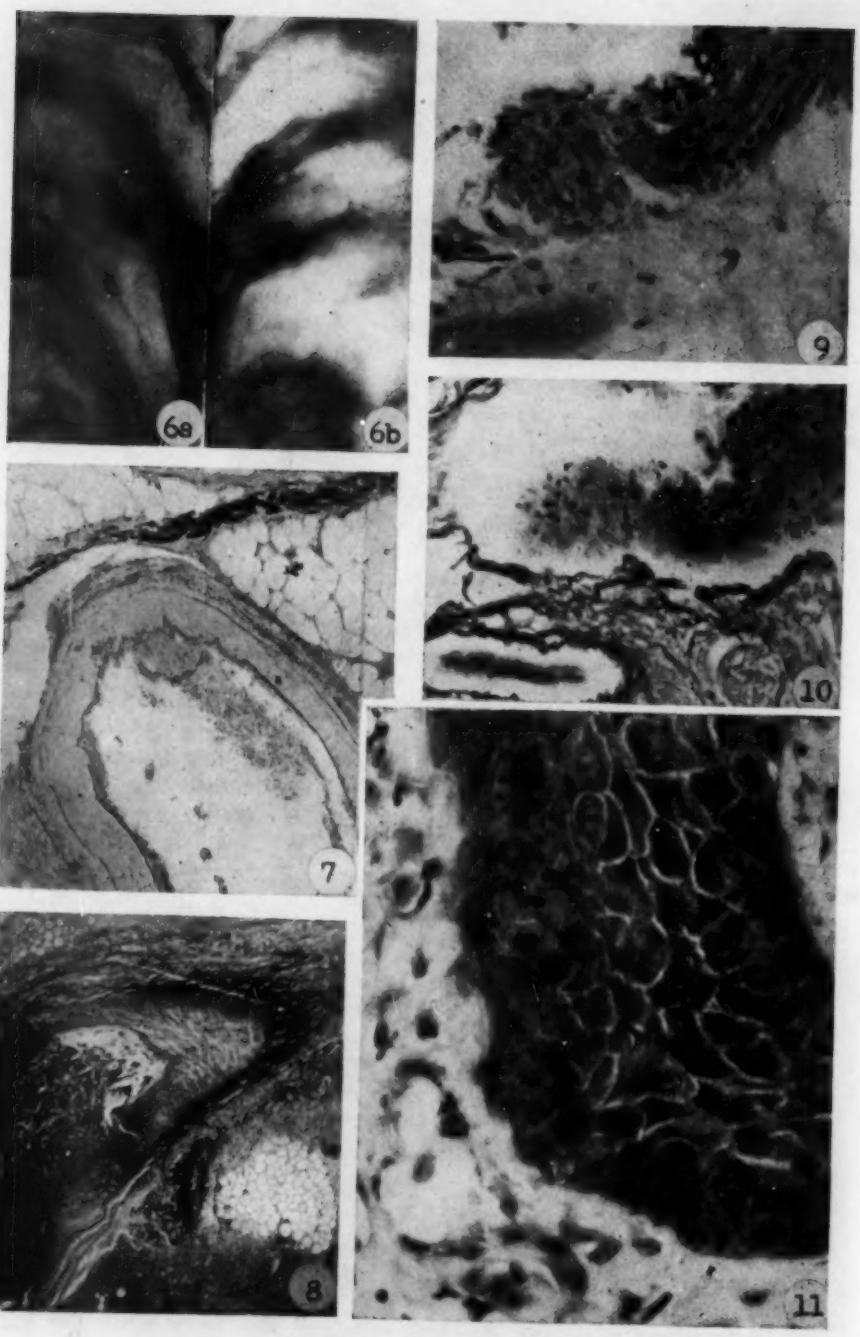
REACTIVE OR REGENERATIVE ZONE

There was a tremendous variation in the extent of pathologic change in this zone from patient to patient and even in the same patient. Entirely normal structures were often found. In no single patient was the whole regenerative process completely represented.

Epidermis.—Three fairly clear areas were seen as the epidermis was traced from the amputation line distally to the zone of demarcation (figs. 1, 9, 10, 11, and 16).

Distally the epidermis in this zone consisted of a few layers of rather flat epidermal cells, with clear intercellular bridges, growing out like flat tongues. There was little differentiation of the stratum granulosum or the stratum lucidum, but a covering of flat squamae was present (fig. 9). This flat epidermal tongue undercut the demarcation zone, tending to separate the gangrenous and demarcation zones from the viable tissue (fig. 1). The basement membrane was clearly demonstrable up to the point where the epidermal "tongue" ended as a few irregular stellate cells (fig. 10). The underlying connective tissue was fibrous and resembled the reticular layer more than the papillary layer (fig. 10). In rare instances the epidermis had grown past the viable tissue and was found resting on a substrate of dead papillary tissue (fig. 10).

Just proximal to the flat epidermal "tongue" was an area with hyperplastic epidermal rete pegs (figs. 1 and 11). The cells were swollen and had numerous mitoses and vesicular nuclei. The mitoses were located in the rete pegs (fig. 11), not in the surface epithelium. Binucleate cells were seen. Some of the cells had paranuclear vacuoles. There was a great deal of variation in the degree of hyperplasia of this area from patient to patient, but in general it varied inversely with



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the amount of collagen in the papillary substrate and directly with the vascularity and edema. The epidermis was always separated from papillary connective tissue by a well defined basement membrane (fig. 24).

The third area, except for mild hyperplastic changes, appeared normal (fig. 16).

Sweat Glands.—There was no clear difference between the secretory and excretory sweat gland cells of the patients and those of the controls except for the degeneration to be described, nor was there unequivocal evidence of any unusual regenerative activity, although in many instances the sweat glands were in areas in which the surrounding structures were in the midst of active regeneration. The total number of sweat glands was within the normal range.

Degenerating ducts were found in about one sixth of the sweat glands. The degeneration was usually found in the superficial third of the reticular layer and did not occur in areas remarkable for either degeneration or regeneration. The degeneration was initiated by cytoplasmic swelling; this was followed by fusion of the cytoplasm of the cells, resulting in a structure resembling a foreign body giant cell (fig. 12). That such structures were not foreign body giant cells was indicated by the observation that at this state inflammatory cells were never found around the sweat glands with these large cells. There was no degenerating material to attract giant cells, and later, when degeneration had set in, only neutrophils were attracted (fig. 13). Fibers were related to the pseudo giant cell in such a way as to suggest a basement membrane separating epithelium from connective tissue.

The next stage of the degeneration was marked by the appearance of large, irregular granules staining similarly to the keratohyaline granules of the epidermis. Finally, pyknosis of nuclei and hyaline clouding of cytoplasm ensued, coinciding with an infiltration of neutrophilic leukocytes.

The somewhat myxomatous periglandular areolar tissue about the secretory acini not only was devoid of inflammation but was distinctly vascular. The edema was external to the reticular fiber basement membrane but had penetrated the collagenous fiber capsule of the sweat gland acini. In certain patients the elastic fibers of the gland capsules were decreased in number. An abnormal pattern

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Fig. 6.—Serial sections of a sclerotic, partially mummified nerve with good preservation of histologic structure. In *b* the collagen stained red and blue, indicating partial mummification. ([*a*] Hematoxylin-eosin-azure II; × 1,100; Army Institute of Pathology negative 98489. [*b*] Mallory-azocarmine; × 1,100; Army Institute of Pathology negative 98487.)

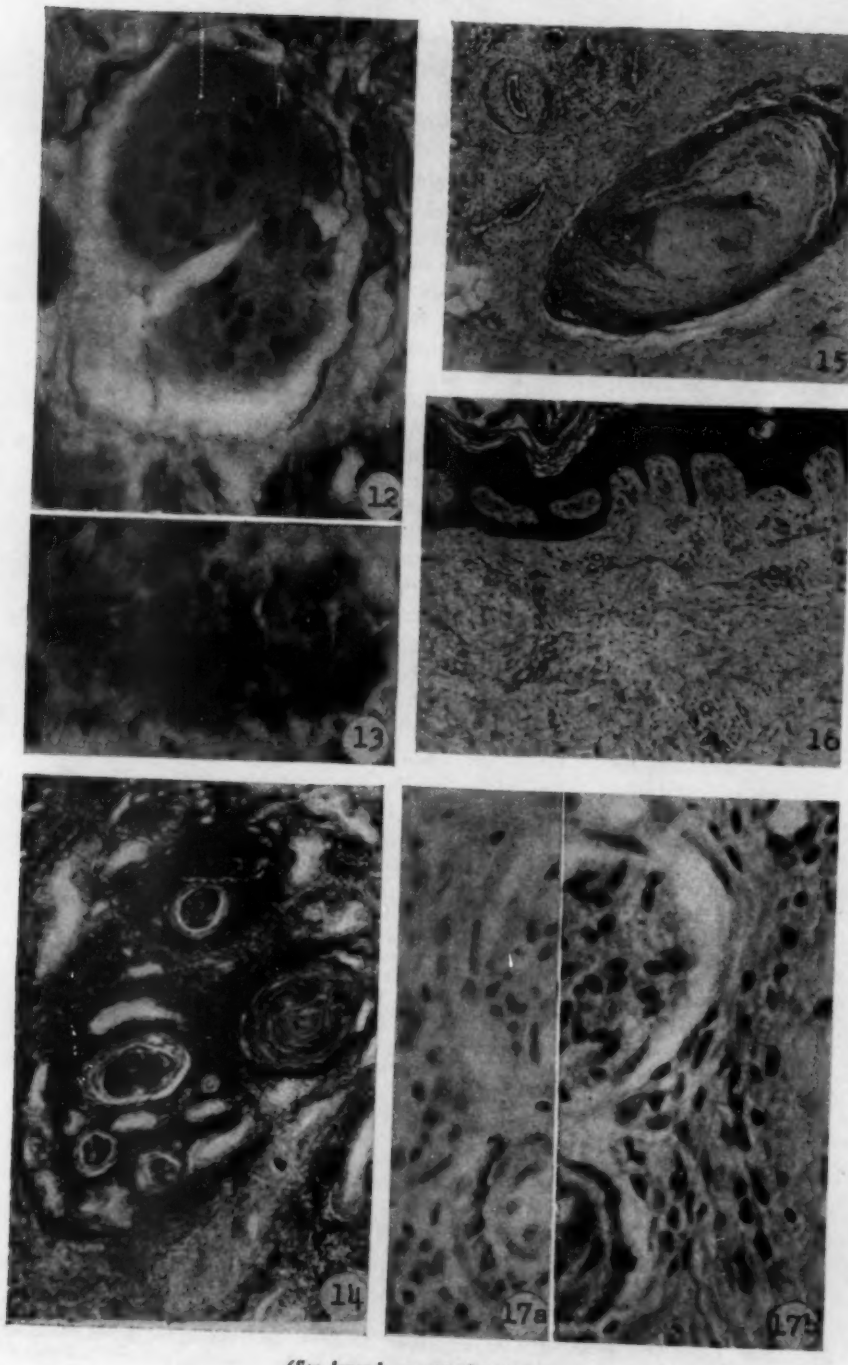
Fig. 7.—Distended subcutaneous artery in the gangrenous zone, with flattened, irregular, discontinuous internal elastic membrane. (Orcein; × 70; Army Institute of Pathology negative 98488.)

Fig. 8.—Junction of the gangrenous and reactive zones passing through an artery. Note the sclerosis around the fat lobules at the lower right. (Mallory-azocarmine; × 26; Army Institute of Pathology negative 98514.)

Fig. 9.—High power view of the distal tip of a flat epidermal "tongue" extending from the right to grow over the avascular papillary substrate. (Hematoxylin-eosin-azure II; × 210; Army Institute of Pathology negative 98495.)

Fig. 10.—Serial section next to that shown in figure 9. The reticular fiber basement membrane is present under the advancing epidermal "tongue" up to the very end of the epidermis. (Silver; × 210; Army Institute of Pathology negative 98493.)

Fig. 11.—Rete pegs in a hyperplastic area of the epidermis, with swollen, hypertrophic cells and numerous mitoses resting on a vascular, mildly inflamed, and edematous papillary layer. (Hematoxylin-eosin-azure II; × 500; Army Institute of Pathology negative 98472.)



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of elastic fibers was not invariably present in the glands with the most surrounding edema and, in addition, was seen in glands with little surrounding edema.

Nerves.—Generally there was little evidence of pathologic change in the nerves, and often it was limited to edema. It was not unusual to see a normal nerve passing through an area of inflammation. Occasionally, fatty macrophages were seen between nerve fibers or within a nerve. The Schwann cells were a little decreased in number. Most spectacular, when present, was extreme sclerosis, which was so intense that the nerves resembled the glomeruli of chronic glomerulonephritis. The fibrosis began as a thickening of the fine collagenous and reticular endoneurial fibers normally present and progressed to become a mass of wavy connective tissue fibers with an occasional Schwann cell. This then contracted to give the final picture (fig. 14). Vascularization of these areas of whorled neural fibrosis was rarely seen (fig. 15). The neural fibrosis seemed to appear most often in the larger, deeper nerves, but this impression may have been due to the difficulty of recognizing the smaller nerves. No evidence of regeneration (mitosis, increase in number of Schwann cells, cellular hypertrophy) was seen.

Arteries.—The great majority of the arteries were normal. In general, the less change in the surrounding tissue, the more normal were the vessels. The most common pathologic feature was edema, which was part of the generalized edema of the neurovascular septums (fig. 16). In the larger arteries it was most noticeable in the adventitia. A distortion of the normal regular vascular pattern was often seen, especially in the subpapillary areas (fig. 16). The collagen and the reticulum were occasionally thickened, primarily at the inner edge of the media. The internal elastic membrane was sometimes discontinuous in the smaller arteries (fig. 17*a* and *b*); less often new elastic fibers had been deposited on its inner surface, and very occasionally it was shredded. An increase of elastic fibers could be observed in the media in a few instances. In general, proliferative changes of elastic fibers were rare. The arteries were seldom invaded by inflammatory cells and, unlike those in thromboangiitis obliterans, were not matted together with the vein.

By studying serial sections, it was usually possible to demonstrate a sludge of agglutinated red cells distending the lumens of arteries in which the processes

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Fig. 12.—Fine collagenous fibers separate the pseudo giant cell from the surrounding connective tissue. (Mallory-azocarmine; × 660; Army Institute of Pathology negative 98502.)

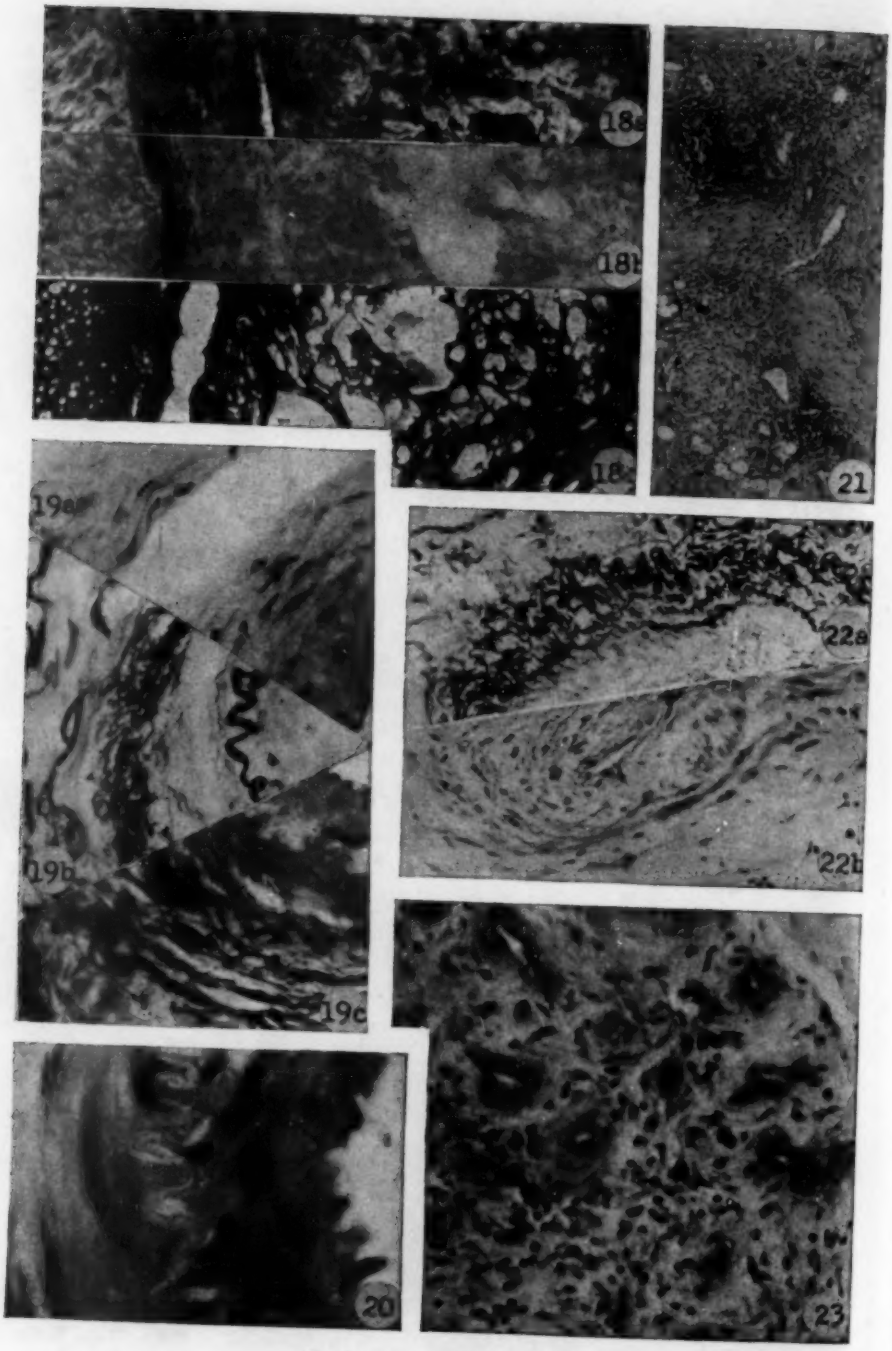
Fig. 13.—Final stage of the destruction of sweat gland ducts with an infiltration of polymorphonuclear leukocytes. (Hematoxylin-eosin-azure II; × 550; Army Institute of Pathology negative 98499.)

Fig. 14.—Stages of the fibrosis of nerves brought about by thickening of endoneurial collagen. The most scarred nerve is at the middle left; the least scarred, at the middle right. (Mallory-azocarmine; × 70; Army Institute of Pathology negative 98835.)

Fig. 15.—Vascularization of an extremely sclerotic nerve. (Hematoxylin-eosin-azure II; × 55; Army Institute of Pathology negative 98501.)

Fig. 16.—Mildly regenerative area with fairly normal epidermis and rete pegs. Note the expansion of neurovascular septums due to edema, mild inflammation and increased number of vessels. (Hematoxlyin-eosin-azure II; × 48; Army Institute of Pathology negative 98833.)

Fig. 17.—Recanalized small artery in a mildly inflamed neurovascular septum. Note the original internal elastic membrane, irregular, discontinuous and flattened, between the new and the original media. [*a*] Orcein; × 600; Army Institute of Pathology negative 98630. [*b*] Hematoxylin-eosin-azure II; × 600; Army Institute of Pathology negative 98669.)



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of organization and canalization had not progressed. Occasionally the sludge was found to be invaded by granulation tissue (fig. 18). From the very beginning, reticular and collagenous but never elastic fibers were found in this rather vascular granulation tissue. Granulation tissue was but rarely seen invading the red cell sludge in a vessel whose walls were dead. The vascularity of this invading tissue was much greater that that found ordinarily in primary intimal proliferation. The internal elastic membranes were usually flattened (fig. 18*b*), indicating that the process had been initiated in vessels that were once paralytically dilated. It was often difficult to recognize the smaller arteries at this stage, because the media was irregular, but a study of the fiber preparation, especially the elastic fibers, in the serial sections was usually helpful.

The further development of the canalization consisted of a swelling of the fibroblasts of the granulation tissue and differentiation of some of them into smooth muscle cells (figs. 19 and 20). In one section this occurred about one of the capillary loops while the original lumen was still filled with agglutinated red cells. Characteristically, these new muscularis cells were swollen and epitheliod in appearance and had poorly developed reticular and collagenous support (fig. 20). An internal elastic membrane had not formed inside this new muscularis (fig. 19*b*). Fibrosis had often occurred in the original muscularis (fig. 19*c*). The internal elastic membrane was usually not thickened or split. A patch of myxomatous change was sometimes seen in the remains of the original intima, and all the other changes previously described (edema, dilatation) were also found. The media was replaced by granulation tissue with iron-containing macrophages in some of the arteries adjacent to the demarcation zone.

In 2 instances a special type of degeneration was seen, consisting of the replacing of the smooth muscle cells by a mass of azocarminophilic granules. This occurred only adjacent to the demarcation zone where an otherwise normal artery would be found ending abruptly at the zone. The fiber pattern of these vessels was normal.

Veins.—A surprisingly high percentage of the veins were also normal, especially of those distant from the demarcation zone, where the other pathologic changes

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Fig. 18.—Serial sections of a large artery illustrating an early stage of the invasion of the red cell sludge. Elastic fibers are present only in the flattened internal elastic membrane of the original dilated artery. ([*a*] Hematoxylin-eosin-azure II; × 255; Army Institute of Pathology negative 98508. [*b*] Orcein; × 255; Army Institute of Pathology negative 98509. [*c*] Silver; × 255; Army Institute of Pathology negative 98510.)

Fig. 19.—Serial sections of the same recanalized artery with new muscularis inside the original internal elastic membrane. ([*a*] Hematoxylin-eosin-azure II; Army Institute of Pathology negative 98519. [*b*] Orcein; × 315; Army Institute of Pathology negative 98556. [*c*] Mallory-azocarmine; × 315; Army Institute of Pathology negative 98517.)

Fig. 20.—Higher power magnification of the section shown in figure 19 *a*, to illustrate the epithelioid swollen muscle cells of the new media. (Hematoxylin-eosin-azure II; × 900; Army Institute of Pathology negative 98555.)

Fig. 21.—Edematous neurovascular septums with dilated veins and mononuclear inflammatory cells. (Hematoxylin-eosin-azure II; × 62; Army Institute of Pathology negative 98629.)

Fig. 22.—Serial section of a recanalized vein with elastic fibers present only in the original intima-media. ([*a*] Orcein; × 235; Army Institute of Pathology negative 98712. [*b*] Hematoxylin-eosin-azure II; × 235; Army Institute of Pathology negative 98713.)

Fig. 23.—Angiomatous granulation tissue. (Hematoxylin-eosin-azure II; × 225; Army Institute of Pathology negative 98506.)

were likewise minimal or absent. Edema, irregularly of vascular pattern, increase in number and generalized mild dilatation were the changes most often encountered in veins, especially in the more superficial layers (fig. 16). The perivenous mononuclear infiltration varied with the extent of inflammation, and when it was extensive, mononuclears invaded the walls of the vein (fig. 21). In the larger veins fibrosis of both intima-media was often found.

A spectacular recanalization, the labyrinthine endophlebitis of Friedman,[3e] was demonstrable. In the veins one could rarely find, as one could in the arteries, the earliest stage of the process—that is, the invading of the red cell sludge by granulation tissue. Usually what was seen was the distended vein with much of its original lumen filled with granulation tissue. In such instances the orcein preparations were invaluable in demonstrating the site of the original intima-media.

In the most advanced stages (fig. 22) a few scattered smooth muscle cells could be seen about the lumen of the vein, external to that a space filled with myxomatous connective tissue, and finally a network of smooth muscle. Collagenous and reticular fibers were interwoven in the two muscle layers, but elastic fibers were well developed only about the outer original muscle layer. Occasionally, smooth muscle could be distinguished around several capillaries in the proliferated intima of a single vein, and the smooth muscle of the original intima-media was disrupted, so that the true original structure was recognized only by tracing the vessel through the series of different stains. In practically every case in which a few small capillaries and veins were found in close proximity in a myxomatous tissue, a vein was being recanalized.

Connective Tissue Proper.—The connective tissue proper had undergone varying degrees of pathologic change. In general, edema, numerous inflammatory cells and dilated vessels were found under hyperplastic epidermis and near the demarcation zone (figs. 15, 16 and 17); near the amputation line the connective tissue was less active.

In many cases the granulation tissue was so vascular that it resembled an angioma, particularly in areas where there was no evidence of recanalization of preexisting vessels and in close proximity to hyperplastic epidermis (fig. 23). The granulation tissue was vaguely reminiscent of Kaposi's sarcoma. The angiomatous areas were composed of numerous small irregular capillaries with closely adherent perivascular mesenchymal cells. Between the capillaries was a diffuse scattering of mononuclears and fibroblasts. All of the cells were hypertrophic. The perivascular mesenchymal cells bore essentially the same relationship to the capillaries as the embryonic mesenchyme does to the endothelium in the differentiation of arteries and veins. Elastic fibers were never found closely related to the newly formed capillaries in these angiomatous areas, but each capillary was surrounded by a network of reticular and collagenous fibers.

Papillary Layer: Mild edema was found, as well as slight but universal dilatation of the capillary loops (figs. 11, 16 and 24). The reticular fibers were teased apart by the mild edema (fig. 24), and the elastic fibers were irregular and decreased in number. The basement membrane was always well preserved (fig. 24). Under the more hyperplastic epidermis, the edema, mononuclear infiltration, cellular hypertrophy and capillary dilatation were more marked. In some instances, numerous mast cells were seen developing from fixed macrophages. In general, neutrophils were rare or absent. In 1 case numerous eosinophils were observed. Under the advancing epidermal "tongue" previously described, the papillary tissue was fibrous and avascular (figs 9 and 10).

Reticular Layer.—The most common finding was an expansion of the neurovascular septums, which was caused by edema, an increased number of vessels, fibers and inflammatory cells, and, occasionally, foci of subacute inflammation (figs. 16 and 21). Under low power magnification the reticular layer seemed to be divided into little areas of fibrous tissue, separated from one another by the hyperplastic, edematous neurovascular septums. The collagenous fibers between the septums were slightly sclerotic and sometimes appeared as small sclerotic whorls between the neurovascular septums. The elastic fibers were irregular, and in the more actively inflamed areas they were entirely absent. Even in the tissue sections in which pathologic changes were minimal, edema and vasodilatation were present.

Subcutaneous Layer.—The changes in this layer were identical with those in the reticular layer except for the presence of the fatty tissue (fig. 25). The interlobular fat septums were expanded in the same manner as the neurovascular septums of the reticular layer. Intercellular edema was marked and mononuclear infiltration minimal. Epithelioid masses of fatty macrophages, resembling fetal fat, often separated the fat cells, but it was possible, as in the bone marrow, to demonstrate transitional forms from inflammatory mononuclears (polyblasts of Maximow) to numerous vacuolated fatty macrophages. The polyblast cytoplasm hypertrophied and became vacuolated while the nucleus became lighter and the chromatin more delicate (figs. 29 and 30). Also, the reticular and collagenous fibers were arranged around the fat cells in such a way as to suggest that the fatty macrophages had penetrated between the fat cells and were not derived from the latter, since each fatty macrophage did not have its own reticular fiber sheath as did the fat cells (fig. 25).

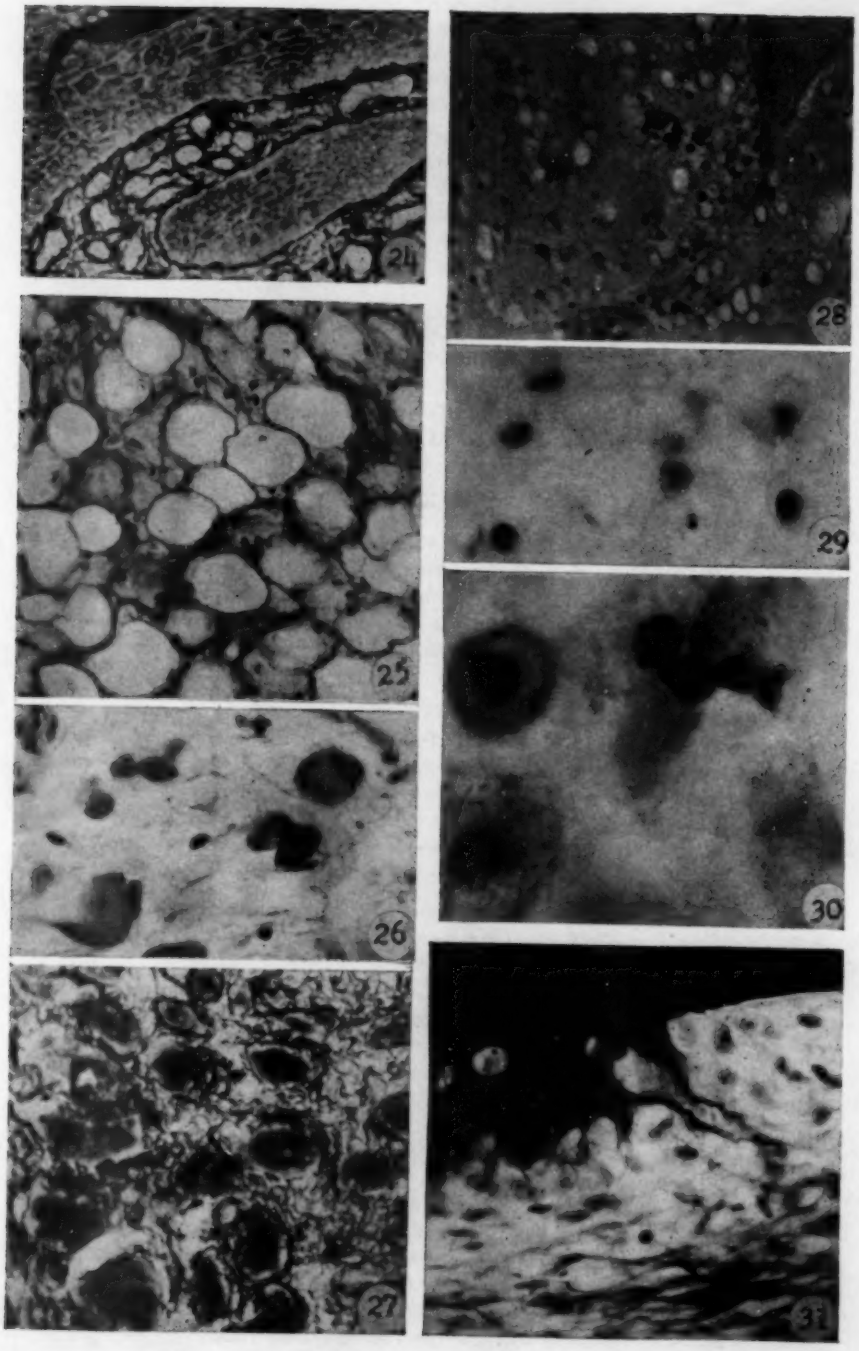
Striated Muscle.—Where there was extreme hyperplasia of muscle nuclei, the normal cytoplasmic striation was indistinct. Many of the nuclei were swollen and lobate. In some areas there were long successions of fibers with excessive numbers of nuclei. There was no evidence that inflammatory cells invaded the muscle fibers (fig. 26). The reticular and collagenous fiber sheaths were normal, but the fibers were separated by an edematous tissue with sparse reticular and collagenous fibers and inflammatory mononuclears (fig. 27).

Bone and Marrow.—In general, there were two types of marrow in the reactive zone, the somewhat fibrous marrow adjoining the gangrenous tissue and the aplastic marrow at some distance from the gangrenous tissue.

In the latter type there was gelatinous degeneration of the marrow similar to that of the aplastic stage which follows irradiation and nitrogen mustard therapy (fig. 28). The marrow was replaced by a basophilic colloid-like material. The smaller vessels were distended with red blood cells. Although no lesion was seen in the vessel walls, some intact red cells were scattered diffusely through this gelatinous marrow.

The predominant cell in the gelatinous aplastic marrow was a fibroblast-like reticular cell, cytologically identical with the reticular cell of the bone marrow. Because of the marked decrease in free hemopoietic cells (fig. 28), these reticular cells were prominent, although there was no evidence of an absolute increase in their number. There were also among the reticular cells occasional fixed macrophages, a few of which had numerous fat vacuoles. The osteoblasts were numerous and lined most of the bone spicules but were probably within numbers normal for the age of the patients. Some of the osteocytes appeared viable.

The free cells were primarily mononuclear inflammatory cells (hypertrophied lymphocytes and monocytes, free macrophages, plasma cells) and fatty macro-



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phages of the type seen in the subcutaneous fat lobules. These macrophages, as in the subcutaneous tissues, were derived from the mononuclears by hypertrophy and fat storage in the latter (figs. 29 and 30). No hemocytoblasts or erythroblasts and only a few isolated megakaryocytes were seen. There were a few scattered small eosinophilic and neutrophilic myelocytes with dark nuclei, not resembling too closely the normal human myelocytes. The usual transitional forms connecting the myelocytes to hemocytoblasts were missing. Collagenous and reticular fibers were essentially normal.

Gelatinous degeneration, vessels distended with erythrocytes, and hemopoietic aplasia were also found in the more fibrous marrow, but the collagenous and reticular fibers, as well as the free and fixed lipophages, were much more numerous. In a sense this part of the marrow corresponded to a demarcation zone. The osteoblasts here were also numerous but were unusual in that they were surrounded by a dense feltwork of collagenous fibers, continuous both with that of the marrow and with the matrix of the adjoining bone spicules (fig. 31). It was not possible to determine whether these collagenous fibers were calcified, since no specific staining for calcium was done. Many of the osteoblasts were in the process of being surrounded by the new collagen and so becoming osteocytes. Characteristically, this new osteoid tissue was laid down on the old necrotic bone without any evidence of preceding resorption of necrotic bone.

COMMENT

GANGRENOUS ZONE

Although there has been general recognition of the presence of three general types of pathologic change corresponding to the gangrenous, demarcation and reaction zones seen grossly, little attention has been paid to the significance of the functions of each zone. Reference has been

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Fig. 24.—Numerous dilated vessels, normal basement membrane, and reticular fibers teased apart by edema in the papillary layer under hyperplastic epidermis. (Silver; × 295; Army Institute of Pathology negative 98709.)

Fig. 25.—Pale lipophages in the upper center; reticular fibers investing individual fat cells at the lower left. (Mallory-azocarmine; × 440; Army Institute of Pathology negative 98705.)

Fig. 26.—Multinucleated hyperplastic muscle cells with absence of striations. (Hematoxylin-eosin-azure II; × 400; Army Institute of Pathology negative 98606.)

Fig. 27.—Normal perimuscular fibrous reticulum with increased number of intermuscular reticular fibers and intermuscular edema. (Silver; × 395; Army Institute of Pathology negative 98825.)

Fig. 28.—Gelatinous aplasia of marrow, dilatation of vessels and scattering of mononuclear cells. (Hematoxylin-eosin-azure II; × 210; Army Institute of Pathology negative 98475.)

Fig. 29—Stages of the process by which lymphocytes are transformed into fatty macrophages. The cell showing the least hypertrophy is at the lower left. (Hematoxylin-eosin-azure II; × 660; Army Institute of Pathology negative 98828.)

Fig. 30.—End stage of the formation of fatty macrophages. These cells are identical with those seen in the fatty subcutaneous tissue. (Hematoxylin-eosin-azure II; × 1,100; Army Institute of Pathology negative 98827.)

Fig. 31.—New bone forming around osteoblasts. The necrotic bone at the top was stained red by the azocarmine, contrasting with the blue-stained viable collagenous fibers. (Mallory-azocarmine; × 400; Army Institute of Pathology negative 98476.)

made to the variation in pathologic change from zone to zone,[8] but only Wieting,[31] Staemmler[9] and Siegmund[10a] have emphasized that most of what is seen in the reaction zone is not the true change resulting from exposure to moist cold, and they advised that the early acute stage of trench foot be studied to determine the true pathologic features of trench foot. Siegmund[10] expressed the belief that the reaction zone, to which by far the greatest attention had been paid, represented a nonspecific reaction to injury. Probably, however, it represents a mixture of the primary degenerative and secondary reparative processes. The gangrenous zone, on the other hand, represents the true structure of the tissues at the time they undergo mummification, preserved by desiccation.

Only rarely has tissue been available from trench foot or immersion foot in the early stages.[11] Although there are minor differences, the descriptions are essentially similar. In particular, the pathologic changes observed in the acute stages in Friedman's series[3e] closely resemble those found in the gangrenous zone in the present study and those observed in the same zone by Kriege[12] over fifty years ago. The diffuse degeneration and mummification with loss of differential staining qualities of the gangrenous zone combined with preservation of the gross histologic structure have been noted.[13] Adami and Nichols[14] and Ziegler[15] have separated the primary gangrenous degeneration from the secondary desiccation of the gangrenous tissues. The cells are much more susceptible than the fibers.

Although the normal fiber pattern was usually fairly well preserved, the staining qualities of the collagenous fibers, and in consequence presumably their chemical and/or physical condition, had changed. Gen-

8. (*a*) Hodara, M.: Monatsh. f. prakt. Dermat. **22**:445, 1896. (*b*) Böttcher, H.: Virchows, Arch. f. path. Anat. **312**:464, 1944; (*c*) Hellmuth, M.: Arch. f. klin. Chir. **158**:702, 1930. (*d*) Patterson and Anderson.[3d]

9. Staemmler, M.: (*a*) Virchows Arch. f. path. Anat. **312**:501, 1944; (*b*) Zentralbl. f. Chir. **69**:1757, 1942.

10. Siegmund, H.: (*a*) Zentralbl. f. Chir. **70**:1558, 1943; (*b*) Jahresk. f. ärztl. Fortbild. **34**:439, 1943.

11. (*a*) Ducuing, J.; D'Harcourt, J.; Folch, A., and Bonfill, J.: J. de chir. **55**:385, 1940. (*b*) Blackwood, W.: Brit. J. Surg. **31**:329, 1944. (*c*) Friedman.[3e,f] (*d*) Wieting.[31] (*e*) Staemmler.[9]

12. Kriege, H.: Virchows Arch. f. path. Anat. **116**:64, 1889.

13. (*a*) Hecht, V.: Wien. med. Wchnschr. **65**:1487, 1915. (*b*) Lange, K., and Boyd, L.: Surg., Gynec. & Obst. **80**:346, 1945. (*c*) Greene, R. J.: J. Path. & Bact. **55**:259, 1943. (*d*) Friedman.[3e] (*e*) Hellmuth.[8c] (*f*) Ducuing and others[11a] (*g*) Kriege.[12]

14. Adami, J. G., and Nichols, A. G.: Principles of Pathology, Philadelphia, Lea & Febiger, 1908-1909, vol. 1.

15. Ziegler, E.: General Pathology, translated by A. H. Buck, New York, William Wood and Company, 1895.

eralized fibrosis of tissue has been noted,[16] but whether reference was made to the gangrenous zone specifically has not been clear. The slight changes occurring in elastic fibers have been described by Hodara.[8a] Attention has not been directed to the remarkable resistance of the reticular fibers and in particular to the resistance of the reticular fiber basement membrane.

That degenerative changes do not become complete until about ten days after the vasoconstrictive phase has been borne out in these cases by the interval which elapsed before the appearance of gross gangrene.[17] The delay between exposure and death of the tissues was sufficient for deposition of collagen, the only proliferative change seen, to have occurred in the nerves (fig. 14). Siegmund[10a] proposed that the slowing of metabolic processes was the basic reason for the slow onset of degeneration after the vascular damage in trench foot. This thesis is supported by the observation that cooling of anemic tissues will delay the onset of degeneration[18] or inflammation.[19]

By an experimental approach[20] it has been shown that cooling a limb readily incites the hemolysis that was so evident in this study and in others.[21]

Numerous investigators have studied the early reactions of experimental animals subjected to cold as a substitute for the human reactions of the acute primary stage of trench foot. However, many of them[22] have used acute application of extreme cold rather than chronic exposure to temperatures about the freezing point in a moist environment.[23] It is probable that the results of the latter group of investigators

16. (*a*) Marchand, F.: Allgemeine Etiologie, in Krehl, L., and Marchand, F.: Handbuch der allgemeine Pathologie, Leipzig, S. Hirzel, 1908 (*b*) Friedman.[3e,f] (*c*) Siegmund.[10] (*d*) Greene.[13c]

17. (*a*) Nagelsbach, E.: München. med. Wchnschr. **66**:353, 1919. (*b*) Page, M.: Brit. M. J. **2**:386, 1914. (*c*) Berson, R., and Angelucci, R.: Bull. U. S. Army M. Dept., 1944, no. 77, p. 91. (*d*) Sonnenburg, E., and Tschmarke, P., in von Bruns, P.: Neue deutsche Chirurgie, Stuttgart, F. Enke, 1915, vol. 17, p. 1. (*e*) Block.[1] (*f*) Lesser.[3e] (*g*) Ducuing and others.[11a]

18. Brooks, B., and Duncan, G. W.: Ann. Surg. **112**:130, 1940.

19. Bruneau, J., and Heinbecker, P.: Ann. Surg. **120**:716, 1944.

20. Reineboth and Kohlhardt: Deutsches Arch. f. klin. Med. **65**:192, 1899.

21. Kriege.[12] Hecht.[13a] Greene.[13c]

22. (*a*) Uschinsky, N.: Beitr. z. path. Anat. u. z. allg. Path. **12**:115, 1892. (*b*) Rotnes, P., and Kreyberg, L.: Acta path. et microbiol. Scandinav., 1932, supp. 11, p. 162. (*c*) von Manteuffel, Z.: Zentralbl. f. Chir. **29**:65, 1902. (*d*) Rischpler, A.: Beitr. z. path. Anat. u. z. allg. Path. **28**:541, 1900. (*e*) Kriege.[12] (*f*) Lange and Boyd.[13b] (*g*) Greene.[13c]

23. (*a*) Large, A., and Heinbecker, P.: Ann. Surg. **120**:707, 1944. (*b*) Denny-Brown, D.; Adams, R. D.; Brenner, C., and Doherty, M.: J. Neuropath. & Exper. Neurol. **4**:305, 1945. (*c*) Smith, J.; Ritchie, J., and Dawson, J.: J. Path.

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are pertinent to the problem of the genesis of trench foot, although Greene[24] expressed the belief that trench foot is merely a slow development of frostbite.

One must remember that the rat tail (used extensively experimentally) may not be comparable with the human foot, since it is usually impossible to produce severe gangrene in the tail before the animal itself dies of exposure, even though an attempt is made to protect it and just expose the tail.[25] In human trench foot and immersion foot, gangrene occurs long before the organism as a whole succumbs to the systemic effects of cold.

Epidermis and Sweat Glands.—The development of the degenerative hyaline changes of the epidermis and the appendages of the skin has been described.[26] Clinically it was one of the most obvious signs of gangrene. Uschinsky[22a] was the only investigator who thought that the epidermis in immediate proximity to the sweat glands was more susceptible than the rest of this tissue. In the present study the sweat glands appeared to show less intense change than did the epidermis. In experiments on the rat tail Blackwood and Russell,[23d,e] unlike Böttcher,[8b] were unable to demonstrate gangrenous changes in the epidermis.

Siegmund[10] expressed the belief that the intracellular epidermal vacuoles, which most of the authors have described, are artefacts due to thawing, but it is generally accepted that they are manifestations of the early edema or of damage of a milder type.[27] Clinically the vesiculation or the edema is always present at some time.[28] It seems probable that the lack of vesiculation observed in the more mummified epidermis is due to the desiccation which follows the gangrene, producing the pic-

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& Bact. **20**:159, 1915. (*d*) Blackwood, W., and Russell, H.: Edinburgh M. J. **50**:385, 1943; (*e*) **52**:160, 1942. (*f*) Böttcher.[8b] (*g*) Reineboth and Kohlhardt.[20]

24. Greene, R. J.: Lancet **2**:689, 1941.

25. Böttcher.[8b] Blackwood and Russell.[23d,e]

26. (*a*) Davis, L.; Scarff, J.; Rogers, N., and Dickinson, M.: Surg., Gynec. & Obst. **77**:561, 1943. (*b*) Dittrich, O.: Arch. f. Dermat. u. Syph. **157**:1, 1929. (*c*) Fuerst, E.: Beitr. z. path. Anat. u. z. allg. Path. **24**:415, 1898. (*d*) Friedman.[3e] (*e*) Theis.[7] (*f*) Hodara.[8a] (*g*) Böttcher.[8b] (*h*) Hellmuth.[8c] (*i*) Kriege.[12] (*j*) Greene.[13c]

27. Hodara.[8a] Böttcher.[8b] Siegmund.[10] Rischpler.[22d] Uschinsky.[22a] Smith and others.[23c] Davis and others.[26a]

28. (*a*) Osborne, J., and Cowen, J.: Lancet **2**:204, 1945. (*b*) Edwards, J.; Shapiro, M., and Ruffin, J.: Bull. U. S. Army M. Dept., 1944, no. 83, p. 58. (*c*) Ungley and Blackwood.[3b] (*d*) Lesser.[3c] (*e*) Patterson and Anderson.[3d] (*f*) Friedman.[3e] (*g*) White.[3h,1] (*h*) Wieting.[31] (*i*) Wright and Allen.[3m] (*j*) Staemmler.[9a] (*k*) Sonnenburg and Tschmarke.[17d]

ture of mummification seen in the absence of secondary infection (sphacelization of the older authors[29]).

It is significant that Rischpler[22d] and Uschinsky,[22a] who subjected their animals to severe cold, have stressed the direct effect exercised by this cold on the epidermal cells, while Smith, Ritchie and Dawson[23c] and Böttcher[8b] have concluded from experiments which more closely duplicated the process resulting in trench foot that the epidermal degeneration is secondary to the vascular damage.

Nerves.—There is ample evidence of an effect of depression of the environmental temperature on the function[30] and the structure[31] of the nerves. Bickford[30a] and Denny-Brown and associates[23b] have demonstrated by functional tests that the various functional modalities are selectively inhibited at different temperatures. Furthermore, Denny-Brown has demonstrated the early involvement of the function and the structure of the myelinated nerves as well as the resistance of the fine vasoconstrictor unmyelinated sympathetic fibers. The conclusion drawn from his work furnish an excellent explanation for the observation that in the stage of reactive dilatation the vessels are still capable of vasoconstriction on stimulation.[32]

Surprisingly, the fact that the nerves are involved during the acute stage has not even been mentioned by numerous authors.[33] Presumably they did not see any evidence of early changes in the nerves. Smith, Ritchie and Dawson[23c] found only mild edema, part of the generalized edema, in rabbits under conditions closely simulating trench foot. But it must be emphasized that this group, with the exception of Smith, Ritchie and Dawson, did not use specific histologic methods to demonstrate the degeneration of myelin, the swelling of the neuromuscular spindle and the occasional axon degeneration detectable with the more delicate technics. Also, one must remember that the immediate degenerative changes are seen most clearly only in the acute condition or in animal

29. Larrey.[3a] Adami and Nichols.[14] Ziegler.[15]

30. (*a*) Bickford, R.: Clin. Sc. **4**:159, 1939. (*b*) Plaschke, S.: Wien. klin. Wchnschr. **29**:5, 1916. (*c*) Wright and Allen.[3m] (*d*) Berson and Angelucci.[17e] (*e*) Brooks and Duncan.[18] (*f*) Large and Heinbecker.[23a] (*g*) Denny-Brown and others.[23b] (*h*) Edwards and others.[28b]

31. Patterson and Anderson.[3d] Friedman.[3e] Böttcher.[8b] Staemmler.[9a] Large and Heinbecker.[23a] Denny-Brown and others.[23b] Blackwood and Russell.[23d,e]

32. (*a*) Hertzman, A., and Roth, L.: Am. J. Physiol. **136**:668 and (*b*) 680, 1942. (*c*) Grant, R. T.: Heart **15**:257 and (*d*) 281, 1930.

33. (*a*) Gruber, S.: Beitr. z. path. Anat. u. z. allg. Path. **84**:155, 1930. (*b*) Grant, R., and Bland, E.: Heart **15**:385, 1931. (*c*) Lewis, T.: ibid. **15**:177, 1930; (*d*) **15**:351, 1931; (*e*) Brit. M. J. **2**:795, 1941 (*f*) Grant.[32c,d] (*g*) Lange and Boyd.[13b] (*h*) Greene.[13c] (*i*) Nagelsbach.[17a] (*j*) Davis and others.[26a] (*k*) Dittrich.[26b]

experiments and not in the regenerative zone, where the processes of degeneration and regeneration are present together. Certainly, the study of the gangrenous zone pointed to extreme involvement of the nerves.

Blood Vessels.—The papers in the literature concerning the effect of depression of the environmental temperature on the vessels are so numerous that specific references need not be made. Blackwood[11b] alone failed to find marked vascular reaction in the 2 cases in which he studied an early stage of immersion foot. Blackwood and Russell,[23d] using the rat's tail under conditions greatly resembling trench foot, have failed to find any vascular lesion, although Böttcher,[8b] after a similar experiment, reported contradictory results. Unfortunately most of the work has been done on vessels in the zone of regeneration or at least on vessels which were already the site of reparative processes. Rarely in regard to human material[34] has attention been directed specifically to the vessels in the gangrenous zone, in marked contrast to the amount of effort expended on those in the regenerative zone. However, primarily as a result of animal experiments, emphasis has been placed on the phase of reactive dilation with the marked vasodilation and engorgement of the vessels caused by stasis of red blood cells.[35] Usually the veins were so distended with red cells that it was impossible to determine whether or not the red cells tended to agglutinate along the valves as described by Friedman[3e] and so give rise to the labyrinthine endophlebitis so often seen in the reaction zone.

The smooth muscle cells are the first component of the vessel wall to undergo hyalinization,[36] a fact amply borne out by a study of the Mallory-azocarmine slides in this study. As in the connective tissue proper, the collagenous fibers were the first of the various fibers to undergo hyalinization. Von Manteuffel[22c] described degeneration and new building of elastic fibers in arteries, but from his brief description it is difficult to determine to what extent these processes progressed, and whether he was referring to the gangrenous or to the regenerative zone.

Muscle.—The early injury of striated muscle is manifested mainly by a spotty, irregular change in the cross striations,[37] which is then replaced by irregular hyalinization of the striated muscle fiber.[38] Necrosis occurs

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34. Block.[1] Friedman.[3e,f] Kriege.[12] Hecht.[13a] Rischpler.[22d] Smith and others.[23c]

35. Friedman.[3e] Hodara.[8a] Böttcher.[8b] Hellmuth.[8c] Siegmund.[10] Lange and Boyd.[13b] Greene.[13c] Uschinsky.[22a] Rotnes and Kreyberg.[22b]

36. Kriege.[12] Rischpler.[22d]

37. Staemmler.[9a] Siegmund.[10a] Blackwood.[11b] Rischpler.[22d] Large and Heinbecker.[23a] Smith and others.[23c]

38. Blackwood.[11b] Uschinsky.[22a] Blackwood and Russell.[23d,e]

in some cases but is always patchy.[39] Atrophy of the individual fibers is a comparatively late change.[40] Most of these investigators have described early intermuscular edema. In contrast to the marked susceptibility of striated muscle to cold observed in the rat tail,[25] only slight susceptibility was noted in striated muscle of the rabbit foot.[23c] It seems probable that, because of the patchy, irregular nature of the degenerative change, it was not due to the direct effect of an external physical agent. Judging from the preservation of the intermuscular fibers, one concludes that the muscle cytoplasm must have been far less resistant than the connective tissue fibers.

Bone, Bone Marrow and Cartilage.—In spite of the extreme susceptibility of the marrow, there has been little in the literature concerning the degree to which bone and marrow are involved in cold injury of any nature. Friedman,[3e] in the one bone available to him from a trench foot in the early stage, found no abnormality. Among the very early workers,[41] only Rischpler[22d] gave a complete account. He described nuclear degeneration, dilated vessels and mononuclear infiltration of the marrow as an early response to cold. Blackwood and Russell[23d,e] did not even mention the bone or the marrow, whereas Böttcher[8b] observed in the same material (rat tail), and Siegmund[10b] in man, essentially the same changes as did Rischpler[22d] in the mouse tail.

Mechanism of Development of Grangrene.—It has been clearly established that wet cold is distinctly more injurious than dry cold and, further, that immobility, gravity, air currents, interference with the venous return, trauma, debility, localized pressure and antecedent peripheral vascular disease are all factors predisposing to a more severe form of the disease.[42] Brahdy,[43] in a study of frostbite in New York, was not convinced that humidity was a factor in the severity of cold injury, but, since most of his patients were exposed at below freezing temperatures, moisture could have had little effect. Recently, in recognition of the well known variation in susceptibility of individuals to wet cold, Silverman[2b] and Osborne and Cowen[28a] have suggested that the person with a labile vasomotor system is more likely to have trench foot than more normal persons.

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39. Ungley and Blackwood.[3b] Böttcher.[8b]

40. Patterson and Anderson.[3d] Friedman.[3e] Siegmund.[10b]

41. (*a*) Ribbert, H.: Deutsche med. Wchnschr. **35**:2036, 1909. (*b*) Rischpler.[22d] (*c*) von Manteuffel.[22c]

42. (*a*) Bigelow, W. J.: Canad. M. A. J. **47**:529, 1942. (*b*) Greene, R.: Lancet **1**:303, 1940. (*c*) Osler, W.: ibid. **2**:1368, 1915. (*d*) Block.[1] (*e*) Patterson and Anderson.[3d] (*f*) Friedman.[3e] (*g*) Böttcher.[8b] (*h*) Smith and others.[23c] (*i*) Greene.[24]

43. Brahdy, L.: J. A. M. A. **104**:529, 1933.

Prolonged severe depression of the temperature to — 6C. (21.2F.) exerts a direct lethal effect on the tissues,[44] but that is hardly applicable to the problem of trench foot or immersion foot. Therefore, von Manteuffel's[22e] belief that injury due to wet cold is a result of the direct effect of cold is not pertinent to trench foot. The spotty, discontinuous nature of the degenerative process noted in this study and by Friedman[3e] and Siegmund[10b] militates against the probability of an external physical agent exerting its effect on the deeper tissues. The genesis of trench foot is somewhat more complicated.

The early physiologic responses to the degrees of depression of temperature in trench foot and immersion foot have been most carefully studied by the London school under Sir Thomas Lewis,[45] who have clearly shown that, besides the well recognized vasoconstriction, reactive vasodilation may occur, even while the part is exposed to lowered environmental temperature, and that this vasodilation invariably occurs after the part has been warmed. They have further shown that these primary effects are probably mediated through an axon reflex and are related to the release of an "H" or histamine-like substance and that the sympathetic fibers do not participate in the reaction at any phase. These results have in general been corroborated by Hertzman and Roth,[32a,b] who also have shown that the vasodilator phase is not due to paralysis of the sympathetic vasoconstrictor phase and that the arteries and arterioles are capable of constricting even during the reactive vasodilation phase. The observations of Denny-Brown and associates[23b] that the sympathetic nerve fibers are the most resistant of all nerve fibers indicate that the nerve fibers responsible for transmitting vasoconstrictor impulses are probably intact in experiments simulating trench foot.

Regardless of their ability to contract in trench foot, for some reason the vessels do not contract, and reactive vasodilation with edema and transudation is clinically[46] and microscopically[47] demonstrable. Fell and Hanselman,[46a] in particular, demonstrated that the transudate may be

44. Lake, N. C.: Lancet **2**:557, 1917.

45. Grant.[32e] Grant and Bland.[33b] Lewis.[33c,d,e]

46. (*a*) Fell, E., and Hanselman, R.: Ann. Surg. **117**:686, 1943. (*b*) Ungley, C. C.: Lancet **1**:681, 1943. (*c*) Riehl: Wien. klin. Wchnschr. **28**:294, 1915. (*d*) Ungley and Blackwood.[3b] (*e*) Lesser.[3c] (*f*) Patterson and Anderson.[3d] (*g*) Friedman.[3e] (*h*) White.[3h,i] (*i*) Wieting.[3l] (*j*) Leriche and Kunlin[3n] (*k*) Staemmler.[9] (*l*) Berson and Angelucci,[17c] (*m*) Large and Heinbecker.[23a] (*n*) Davis and others.[26a] (*o*) Osborne and Cowen.[28a] (*p*) Edwards and others.[28b] (*q*) Gruber.[33a]

47. Friedman.[3e,f] Hodara.[8a] Böttcher.[8b] Kriege.[12] Greene.[13c] Adami and Nichols.[14] Rischpler.[22d] Marchand.[16a] Uschinsky.[22a] Smith and others.[23c] Greene.[24] Davis and others[26a] Gruber.[33a] Bigelow.[42a] Greene.[42b]

extensive enought to affect the dynamics of the circulation. In spite of this obvious evidence, some investigators[48] have not mentioned the occurrence of a transudate.

Since the discovery of the arteriovenous anastomoses a great deal of work has been done on their anatomy and physiology,[49] and there can be little doubt of their importance in relation to the control of the temperature and the blood vessels of the extremities. Unfortunately, in spite of the relatively large volume of study, primarily in the form of acute experiments, one must agree with Friedman's[3e] statement: "A satisfactory detailed anatomic study of the arteriovenous anastomoses in lesions produced by cold has not yet been reported."

When one attempts to correlate the controversial literature on the genesis of the lesions in trench foot and allied diseases, the various theories fall into certain rough categories. One group of investigators is of the opinion that the vascular, epithelial, muscular and connective tissue involvement is secondary to a primary direct effect of the cold on the nerves.[50] This theory receives some support from the myelin changes that are readily produced in nerves and from the axonal changes produced with more difficulty.[51] However, even if one accepts the still controversial concept that anatomic evidence of nerve destruction precedes that of the vessels, there still exists the possibility that a functional vascular change is primary to the neural changes. Physiologic proof[32a,b] that practically normal vasoconstrictor impulses are preserved in the presence of marked vascular physiologic reactions would argue against the theory of a primary neural malfunction. It is significant that Denny-Brown and associates[23b] and Large and Heinbecker,[23a] who have worked with the finer neuropathologic technics in conjunction with the study of the functional capacity of the nerves, have maintained that the lesions are such as to suggest primary vascular dysfunction.

The adherents of the concept of a primary vascular lesion may be divided into the group advancing the theory of the primary or the sole importance of vasoconstriction[48] and the majority who regard the succeeding vasodilatation, edema and stasis as most important. The latter

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48. Theis.[7] Nagelsbach.[17a]

49. Clara, M.: Ergebn. d. Anat. u. Entwcklngsgesch. **27**:246, 1927. Clark E. R.: Physiol. Rev. **18**:229, 1938. Clark, E. R., and Clark, E. L.: Am. J. Anat. **55**:407, 1934. Popoff, N.: Arch. Path. **18**:295, 1934. Masson, P.: Bull. Soc. franç. de dermat. et syph. (Réunion dermat., Strasbourg) **42**:117, 1935. Harpuder, K.; Stein, I., and Byer, J.: Am. Heart J. **20**:539, 1940. Grant.[32d] Grant and Bland.[33b] Lewis.[33c,e]

50. Blackwood.[11b] Blackwood and Russell.[23d,e]

51. Friedman.[3e] Böttcher.[8b] Staemmler.[9] Blackwood.[11b] Large and Heinbecker.[23a] Denny-Brown and others.[23b] Blackwood and Russell.[23d,e]

in turn may be subdivided into those favoring the absence, or at least the late appearance, of a mechanical vascular obstruction[52] and those who believe that a vascular obstruction is primary to the whole process.[53] As long as there is a mechanical obstruction, it makes little difference whether it is composed purely or partly of erythrocytes. One cannot place much reliance on failure to find thrombi in the reactive zone as proof that they are absent in the acute stages,[33a] since in persons of the young age group exposed to conditions predisposing to trench foot thrombi are quickly organized. Because of their age, it is improbable that atheromatous plaques were the cause of thrombi in these young soldiers.

The material available in this study when correlated with these divergent views favor the following sequence of events: There is a primary vasoconstriction which coincides with numbness and a pale appearance of the foot; vasoconstriction is then replaced by vasodilation and transudation of fluid; the escape of fluid leaves a dense mass of erythrocytes filling the distended vessel and greatly interfering with the circulation of the blood, a process which gives rise to swelling of the foot, a stage lasting several days. Presumably because of toxic changes in the permeability of the vessel walls, some red cells leak out, causing the formation of hemorrhagic bullae. Thereafter, degenerative changes begin to appear, first functional, later anatomic, in the more specialized structures, epidermis, muscle, nerve and marrow. This last process usually occurred about the time of evacuation and probably was related to the increased vasodilation and hemorrhage occurring when the feet were removed from the cold environment.[54] Mummification then ensued. Since practically all soldiers in this series received antitetanus immunizations and parenteral treatment with sulfonamide compounds and penicillin, as well as protection from further injury, there was no tendency toward the secondary infection and toxic states described so vividly by Larrey.[3a] In spite of the obvious structural alterations at this stage, complete functional and pathologic resolution is still possible,[2] dependent solely on the extent of the vascular obstruction.

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52. (*a*) Winiwarter, F.: Arch. f. klin. Chir. **23**:202, 1879. (*b*) Staemmler.[9] (*c*) Rischpler.[22d] (*d*) Smith and others.[23c] (*e*) Gruber.[33a] (*f*) Bigelow.[42a]

53. Block[1] Patterson and Anderson.[3d] Friedman.[3e] Hodara.[8a] Böttcher.[8b] Siegmund.[10] Kriege[12] Lange and Boyd[13b] Greene.[13c] Adami and Nichols.[14] Ziegler.[15] Large and Heinbecker.[23a] Denny-Brown and others.[23b] Davis and others.[26a]

54. Immersion Foot, editorial, Bull. U. S. Army M. Dept., 1943, no. 70, p. 26. White.[3b,1]

REGENERATIVE ZONE

Regeneration of Epidermis and Sweat Glands.—Fundamentally the reparative ability of the epidermis depends on two factors: first, the inherent capacity of the epithelial cells, whether these originate from the sweat glands or from the surface epidermis, to bridge the defect; second, the presence of a substrate of connective tissue offering circumstances favorable to the realization of this latent regenerative capacity of the epithelial cells. In an exhaustive study of the various factors, Bishop[55] emphasized the role of the connective tissue substrate as the usual limiting factor. It seems clear that cold, or the degeneration due to cold, is a sufficient stimulus to incite mitotic proliferation, formation of giant cells, and hypertrophy of the epithelial cells.[56] The deep epidermal papillae with numerous mitoses described by Bishop[55] and by Fuerst[26c] attest to the intensity at which this proceeds. Fuerst studied epidermal cells that were stimulated by mild degrees of cold and traced the formation of giant cells from epidermal cells and the cells of sweat glands in much the same manner as in the present study. He did not observe the degeneration of these giant cells—really parakeratosis of the epithelium of the sweat glands and ducts.

However, attention has not been directed to the variation in the reaction of the epidermis at different distances from the demarcation zone or to the relation of this varied proliferative activity to the structure of the connective tissue substrate. This accounts for the fact that some observers[26c] have noted an increase in epidermal papillae; others,[57] a decrease, and Staemmler,[9] both an increase and a decrease. The present study indicates that both occur and that the epidermis over the edematous, inflamed papillary tissue is the site of proliferation and that the thin, flat epidermal strip nearest the demarcation zone, resting on a fibrous avascular substrate, is pushed along passively. This is clearly in agreement with Bishop's conception of the connective tissue substrate.[55]

The tendency of the epidermal cells to grow out along a viable substrate accounts for the observation that the epidermis grows under the gangrenous tissue, adhering to the still viable connective tissue.[58] This phenomenon would ultimately result in spontaneous amputation and, if the connective tissue substrate is adequate, reepithelization of the defect. The remarkable ability of the epidermal cells of this age group

55. Bishop, G.: Am. J. Anat. **76**:153, 1945.

56. Hodara.[8a] Rischpler.[22d] Uschinsky.[22a] Smith and others.[23c] Dittrich.[26b] Fuerst.[26c]

57. (*a*) Boland, F.; Claiborne, T., and Parker, F.: Surgery **17**:564, 1945. (*b*) White and Warren.[3k] (*c*) Hodara.[8a] (*d*) Siegmund.[19b] (*e*) Rischpler.[22d] Davis and others.[26a]

58. Hodara.[8a] Rischpler.[22d] Uschinsky.[22a]

to regenerate is further proved by the observation that within a few months the epidermis of patients with trench foot who have lost no tissue but have had some gangrene is normal.[59] White and Warren,[3k] on the other hand, in a study made without the use of normal controls, have reported minimal residual changes such as the flattening of the epidermal papillae.

The interrelationships of epithelial cells, on one hand, and the connective tissue and reticular fibers, on the other, have been studied in the embryo in great detail by Alfejew,[60] in the adult by Plenk[61] and in tissue cultures by Maximow.[62] From their observations it is evident that except in early embryonic life the connective tissue forms a basement membrane at all epithelial tissue—connective tissue interfaces, thus separating these two tissues. In the embryo, as the various epithelial invaginations and evaginations occur, this reticular fiber basement membrane is constantly forming, or reticular fibers already present are condensed into a basement membrane separating the epithelial and connective tissue elements. Alfejew[60] and Plenk[61] have stated that silver impregnation is needed to demonstrate these fibers. The same fundamentals hold true in trench foot, since at no time was there any epithelial regeneration without the presence of a normal basement membrane, demonstrable by silver impregnation, separating the epidermis and sweat glands from the connective tissue substrate, even in highly hyperplastic areas.

Nerves.—The earliest response to the initial damage (myelin breakdown and axonal damage) is an inflammatory reaction with phagocytosis of the myelin.[63] This is closely paralleled by Schwann cell proliferation.[64] The initial inflammation is followed by one of two processes, clearing away of debris and complete regeneration, or marked fibrosis of the nerves, dependent on the intensity of the original injury. In the milder involvements the former was usual[65]; in the more severe (amputation cases) the latter,[66] but even then numerous nerves, appeared normal. Probably whatever regeneration occurs is complete within a few months, and those nerves that were too damaged have become fibrotic.

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59. (*a*) Paddock, F.: New England J. Med. **234**:433, 1946. (*b*) Block.[1]

60. Alfejew, S.: Folia haemat. **30**:111, 1924.

61. Plenk, H.: Ergebn. d. Anat. u. Entwcklngsgesch. **27**:302, 1927.

62. Maximow, A.: Ztschr. f. mikr.-anat. Forsch. **17**:625, 1929.

63. Friedman.[3e] Staemmler.[9a] Uschinsky.[22a] Denny-Brown and others.[23b] Blackwood and Russell.[23d]

64. Siegmund.[10b] Rischpler.[22d]

65. Block.[1] Denny-Brown and others.[23b] Smith and others.[23c] Boland and others.[57a]

66. Friedman.[3e] Siegmund.[10b]

A serious problem is not whether there is anatomic evidence of injury of nerves, since this must be the case where extreme fibrosis has occurred, but how much residual damage of function results. Denny-Brown and associates[23b] have shown that where nerve regeneration has occurred without fibrosis, normal nerve function tends to occur. Unfortunately, injury was not severe enough in his experiments to result in such marked fibrosis as is seen in amputated specimens of trench foot. But within a short distance above the amputation line there is no evidence of fibrosis, and presumably these nerves would be capable of normal function.[1]

Vessels.—In general there are only two mechanisms of vascular repair, recanalization of the preexisting vessel and generation of a new vessel. The details of the reparative process, as well as the pertinent literature, have been summarized by Friedman.[3e] What has not been previously emphasized is that even in amputation specimens a large number of vessels seem to be anatomically normal[10b] and that a few centimeters proximal to the demarcation zone vascular lesions are not seen.[67]

The whole process of organization and recanalization of the arteries and the veins is essentially similar to that observed in other vascular occlusions of varied causes.[68] The intimal proliferation, regardless of whether one believes it to be primary or, which is more probable, secondary to a thrombus, is noninflammatory in nature except immediately adjacent to the demarcation zone.[69] This granulation tissue organizing the thrombus originates largely inside the internal elastic membrane.[3e] The minimal changes seen in the arterial media tend to support this thesis. Friedman,[3a,f] Staemmler[9a] and Winiwarter[52a] have pointed out that the endophlebitis begins primarily about the valves. Unfortunately, in the present study the process was too far advanced for one to verify that statement, but the extreme labyrinthine appearance of the lumens seen inside the recanalized veins would tend to support their point of view.

The early development of the collagenous and reticular fibers of the granulation tissue has not been appreciated except by Wertheman.[68a] But Staemmler[9a] has commented on the absence of elastic fibers in the newly developed, organizing granulation tissue. In contradiction to Gruber,[33a] Nagelsbach,[17a] von Manteuffel,[22c] and Hellmuth,[8c] the development of new layers of internal elastic membrane was not noted in this study. The patients in whom this finding is reported to have been made were old men, and so the proliferation of elastic fibers was prob-

67. Block.[1] Boland and others.[57a]

68. (*a*) Wertheman, H.: Virchows Arch. f. path. Anat. **270**:605, 1928. (*b*) von Manteuffel.[22c]

69. Block[1] Friedman.[3e] Siegmund.[10] Ducuing and others.[11a] Gruber.[33a]

ably related to the normal aging process.[70] Winiwarter,[52] who was fortunate enough to study a patient with an eight-year history, described the development of a new internal elastic membrane as demonstrated in the vessels developing in the intima. Judging by the absence of any other reference to this in the literature, one concludes that in the recanalization of the vessels the development of elastic fibers must be a tardy process as opposed to the immediate formation of collagenous and reticular fibers.

The process by which smooth muscle cells are formed from the perivascular fibroblasts has long been known[68a] and is identical with that by which the smooth muscle cells of the vessels of the embryo and of rabbit ear chambers[71] are formed.

It is doubtful if the recanalized vessels are normal physiologically. The marked narrowing of the lumen, the immature appearance of the new smooth muscle cells and the lack of elastic tissue speak against a normal function. But within a short distance proximal to the amputation line normal vessels are found,[59] and even in the amputation specimens many of the vessels seemed anatomically normal. From a purely clinical point of view in this series there must have been adequate circulation of the blood by the time amputation was performed, since in most cases it was done just proximal to the demarcation zone and in some cases pinch grafts were successfully applied to bridge epithelial defects at the distal ends of the stumps.

Connective Tissue Proper.—The typical changes occurring in connective tissue in the late stages of trench foot have been described as edema, moderate mononuclear infiltration, absence of polymorphonuclears, phagocytosis of hemosiderin, serous atrophy of fat, and sclerosis.[72] Edema and mononuclear infiltration, especially the former, are most likely to be found, even in the presence of the mildest type of reaction.

Probably too much attention has been paid to the sclerosis. It is not diffuse but, as pointed out by Friedman,[3e] is patchy, occurring in islands between the neurovascular septums. Characteristically, the inflammatory cells are lymphocytes and monocytes and various mononuclears derived from these cells. In 1 case in this series and 1 case in Friedman's series[3e] plasma cells were found, although in other respects these cases seemed not to differ from the usual.

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70. Hellmuth.[8c] Nagelsbach.[17a] von Manteuffel.[22c]

71. Clark, E. R.; Hitschler, W. J.; Kirby-Smith, H. T.; Rex, R. O., and Smith, J. H.: Anat. Rec. **50**:129, 1931.

72. Lesser.[3c] Friedman.[3e,f] Hellmuth.[8c] Blackwood.[11b] Hecht.[13a] Nagelsbach.[17a] Gruber.[33a] Paddock.[59a]

Studies of milder forms of trench foot[73] have demonstrated that pathologic changes, as seen in biopsy, are slight or nonexistent. In a previous study[1] it has been shown that the connective tissues a few centimeters proximal to the demarcation zone are normal. Even in the amputated tissues, areas with only minimal changes were seen, and one of the purposes of this study is to call to notice the striking tendency toward healing exhibited by the connective tissues in this age group. The angiomatous areas of new vessel formation may also be considered a manifestation of the intense regenerative efforts in this age group.

Except for the observations made by Siegmund,[10] little attention has been paid to the elastic fibers in the connective tissue. The irregularity of elastic tissue is undoubtedly related to the toughness of the tissue and the difficulties of handling the tissues experienced by surgeons operating on these patients. In any event, in trench foot the elastic fibers regenerate much more slowly than any other component.

The lesions of connective tissue seen in chronic pernio bear a startling resemblance to those seen in the regenerative areas of trench foot (McGovern and Wright;[74] Dittrich[20b]). It is hard to understand why in trench foot there is an intense tendency toward healing while in pernio there is a marked tendency toward chronicity. The difference is not one of age, since pernio occurring in the younger age group was described by McGovern and Wright. In general the pathology corresponding to the other clinical syndromes due to exposure to wet cold is similar to that of trench foot.

Muscle Regeneration.—Little may be added to the original description of Blackwood[11b] as far as the histologic appearance of the striated muscle is concerned. He illustrated the residual small atrophic muscle fibers, the marked intermuscular edema and inflammation, the hypernucleated muscle fibers and the loss of cross striation. His findings have been corroborated[75] in all essentials. White and Warren[3k] found in biopsy material that, of all the pathologic phenomena described, only the fibrosis and atrophy of individual fibers were present in mild forms of immersion foot without loss of tissue about four months after exposure. In a carefully written, well illustrated paper, Blackwood and Russell[23e] showed that in the rat at the end of one year the fine terminal myelinated fibers and motor end plates were still slightly abnormal and advanced the thesis that the failure of normal reinnervation is the main cause of the atrophy of striated muscle in the parts exposed to cold.

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73. Block.[1] Boland and others.[57a] Paddock.[59a]

74. McGovern, T., and Wright, I.: Am. Heart J. **22**:583, 1941.

75. Patterson and Anderson.[3d] Friedman.[3e] Böttcher.[8b] Staemmler.[9a] Siegmund.[10b] Blackwood and Russell.[23e]

Bone and Marrow Regeneration.—The marrow and bone have been studied most extensively by Siegmund[10] and Blackwood.[11b] They have described serous-fatty atrophy and a subacute type of inflammation. Blackwood has maintained that the old bone is resorbed in the early stages and that new bone is laid down after four months. However; the present investigation, as well as most others,[76] has clearly demonstrated the new, perhaps still uncalcified bone laid down in apposition to the old, dead bone. Without the use of specific calcium impregnations it was impossible to determine whether this new bone was calcified normally or remained as osteoid tissue for an unusually long time.

Friedman[3e] also noted the phagocytosis of lipoid material but did not discuss the source of the phagocytes. Probably they are derived from both the fixed reticular cells and from the mononuclear inflammatory cells (lymphocytes and monocytes).

The fact that fibrosis was restricted to areas near the gangrenous marrow demonstrates that fibrosis is not a universal sequel to tissue severely damaged by cold. In the more aplastic gelatinous areas even the special fiber preparations failed to reveal any increase in collagenous or reticular fibers.

The aplastic marrow, with gelatinous atrophy, mononuclear infiltration, absence of hemopoiesis, prominent reticular cells and dilated vessels, resembled marrow that is in an aplastic stage after irradiation[77] or after nitrogen mustard therapy.[78] However, it lacked the large amount of phagocytosed iron pigment seen after employment of these therapeutic agents.

The aplastic area also bore a certain resemblance to embryonic marrow as this appears during the transition from the primary to the secondary marrow described by Maximow.[79] Especially noteworthy in both instances are the small dark myelocytes, not derived from hemocytoblasts but from histioid wandering cells in the adult. The presence of myelocytes and the absence of erythroblasts are similar to what is seen in the early secondary stage of embryonic marrow.

Analogous to what has been observed after irradiation of marrow and after nitrogen mustard therapy, the whole process in the marrow may be reversible in that the reticular cells would probably be able to serve as

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76. Friedman.[3e] Böttcher.[8b] Siegmund.[10b] von Manteuffel.[22e] Ribbert.[41a]

77. Bloom, W.: The Histopathology of Irradiation from External and Internal Sources, National Nuclear Energy Series, New York, McGraw-Hill Book Company, Inc., 1948.

78. Block, M.; Spurr, C., and Jacobson, L.: Ann. J. Clin. Path., to be published.

79. Maximow A.: Arch. f. mikr. Anat. **73**:444, 1909.

a source of heteroplastic hemopoiesis. However, one must remember that the marrow in the extremities is usually quite inactive, probably, as Huggins and Blacksom[80] showed in the rat tail, because of the lowered temperature with respect to the rest of the body. Therefore the marrow in these amputated toes was more abnormal because of the myxomatous atrophy than because of a decrease in hemopoiesis.

SUMMARY

Three clear zones of tissue change were usually present in trench foot: gangrene, demarcation and reaction. The gangrenous zone was made up of the tissue which had died and then become mummified early after exposure. Because of the mummification, the tissue in this zone was preserved more or less as it had been at the time of tissue death. The major manifestation of injury and probably the most important cause of the gangrene was the spectacular dilation and occlusion of vessels occurring during the phase of reactive dilation leading to infarction of the more peripheral areas. The essential structure—in particular, the fiber scaffolding of the tissues—was remarkably well preserved in this zone.

The gangrenous and the demarcation zone were essentially nonspecific in nature. They were similar to comparable zones found in any of the bland ischemic necroses. The only special feature was the intensity and speed with which the reparative efforts took place in the reaction zone, presumably due to the youth and the general good health of the patients, as well as to the lack of any antecedent peripheral vascular disease.

The cellular structure of the corium, especially that of the subpapillary layer, was the most important factor governing the epithelization of any defect. A great deal of normal tissue was always present in the reactive zone, especially near the amputation line. Mild edema, slight subacute inflammation, recanalization of vessels, neoformation of vessels and focal hyalinization were the most common findings in this zone. The reticular and collagenous fibers were laid down very early in the reaction to injury, but at the time of amputation, several months after the initial injury, there was still little evidence of comparable regeneration of elastic fibers. Whatever functional impairment was still present in the

80. Huggins, C., and Blocksom, B.: J. Exper. Med. **64**:253, 1936.

reactive zone immediately proximal to the gangrenous tissues was probably related to the residual vascular abnormality, absence of elastic fibers and spotty neural fibrosis. However, within few centimeters proximal to the gangrenous tissue there was no evidence of any residual lesions.

Attention is directed to the present complete ignorance of the pathologic anatomy of the glomus in trench foot and related diseases. Perhaps a study of this structure in both warm-blooded and cold-blooded animals which normally live in cold wet weather without apparent disability would add to the knowledge of this structure and consequently to knowledge of reaction to cold in general.

DEVELOPMENT OF A STATE REFRACTORY TO GROWTH OF A MOUSE TUMOR IMPLANTED IN THE ANTERIOR CHAMBER OF THE GUINEA PIG EYE

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AND
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IN THE COURSE of studies of heterologous and homologous tissues transplanted into the anterior chambers of guinea pig eyes, it was observed that when growth occurred it was followed almost invariably by regression. In the case of a mouse carcinoma, MT8, it was further noted that after this tumor had grown and regressed in one eye of a guinea pig, a relatively refractory state developed. Only rarely was the same tumor successfully transplanted a second time into the same eye or into the opposite eye. The purpose of this report is to record our observations of the development of this refractory state.

The growth of tissues transplanted from alien species to the anterior chamber of the eye described by Greene[1] suggests that this location provides a more favorable environment for heterologous transplants than does the subcutaneous tissue. However, Greene observed that regression is a common, but not invariable, fate of successful intraocular heterologous tumor transplants. Greene[2] also observed that regression of a transplant in one eye may be associated with the development of a state relatively refractory toward the same tumor and sometimes another tumor when these are later transplanted into the opposite eye.

Appel and co-workers[3] studied the relative degrees to which the ocular and the subcutaneous routes resist transplantation of the Brown-Pearce carcinoma in rabbits. They observed that after a subcutaneous growth of this tumor had become established, the animals were resistant to growth of the same tumor when it was retransplanted subcutaneously but were not resistant to growth of intraocular transplants. These authors

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1. Greene, H. S. N.: J. Exper. Med. **73**:461, 1941.
2. Greene, H. S. N.: Cancer Research **2**:669, 1942.
3. Appel, M.; Saphir, O.; Janota, M., and Strauss, A. A.: Cancer Research **2**:576, 1942.

demonstrated a complement-fixing antibody in the blood serums of rabbits which were immune to this tumor. The primary aqueous humor of the immunized animals did not contain the antibody, although the secondary or reformed aqueous humor contained it, in variable amounts.

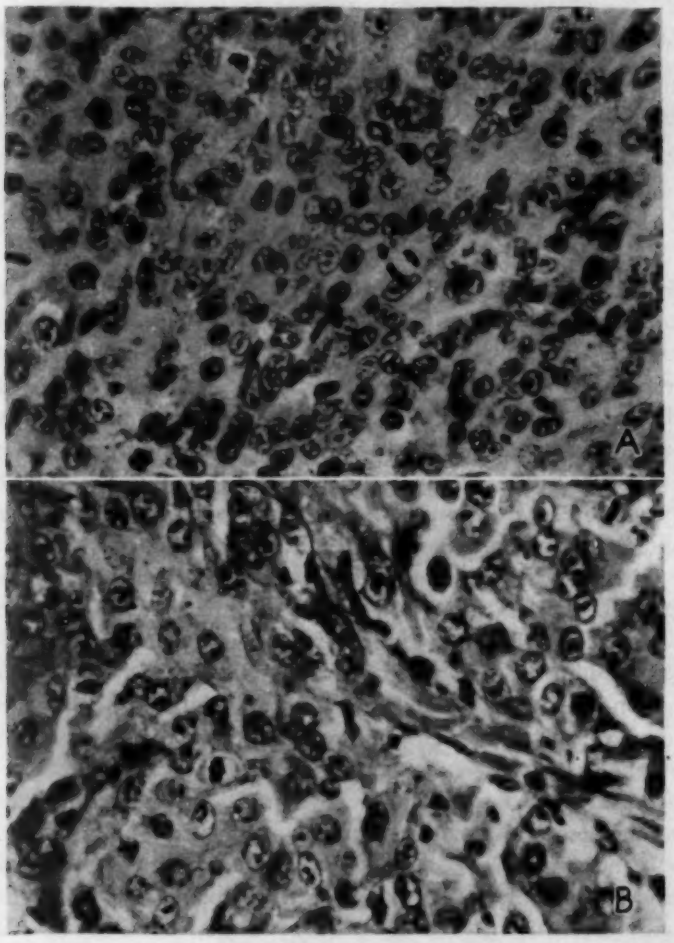

Fig. 1.—*A*, histologic characteristics of mouse carcinoma MT8 before it was transplanted into the anterior chamber of the guinea pig eye.
B, histologic characteristics of the carcinoma after it had been transplanted into the anterior chamber of the guinea pig eye.

Besredka and Bardach,[4] however, failed to obtain growth of intraocular transplants of the Brown-Pearce carcinoma in rabbits previously immunized against this tumor.

4. Besredka, A., and Bardach, M.: Compt. rend. Acad. d. sc. **202**:2193, 1936.

Cheever and Morgan[5] likewise observed that rabbits immunized by any of several routes against the Brown-Pearce carcinoma were refractory to intraocular growth of this tumor. The growing of the tumor in one eye, however, failed to produce a refractory state in the opposite eye.

MATERIALS AND METHODS

For our studies of the regression of intraocular heterologous tumor transplants, we made use of a transplantable bronchogenic mouse carcinoma, MT8, which arose spontaneously in Gardner's laboratory. This tumor was furnished to us by Dr. H. S. M. Greene. The histologic characteristics of this neoplasm before and after it was transferred into the anterior chamber are shown in figure 1. The clinical appearance of the growth in the anterior chamber is shown in

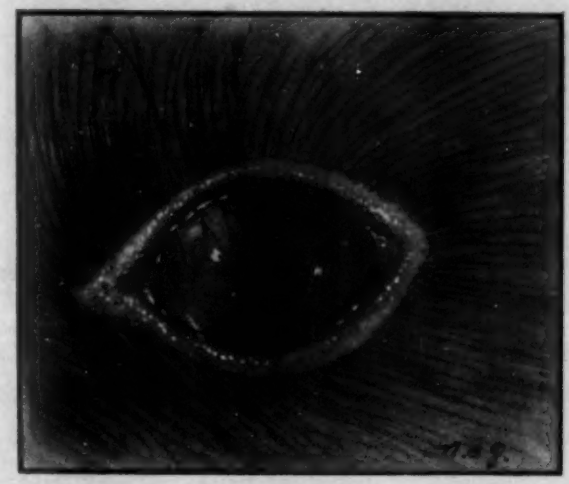

Fig. 2.—Gross appearance of a growing anterior chamber MT8 transplant three weeks after transplantation.

figure 2. The mouse tumor was carried by regular transplantation in the subcutaneous tissue of mice of various strains. The technic of transplantation, although modified in detail, is similar to that described by Greene. Young guinea pigs of both sexes weighing approximately 200 Gm. were used. Anesthesia was induced with an intraperitoneal injection of pentobarbital sodium (6 mg.), supplemented by topical anesthesia of the cornea and the conjunctiva with 2 per cent butacaine sulfate solution. Fragments of tissue 1 to 2 mm. in diameter were removed aseptically from non-necrotic portions of the tumor. The bits of tumor were kept moist in Tyrodes's solution, and representative specimens were set apart for histologic examination. An opening about 3 mm. long was made with a sharp keratome through the cornea into the anterior chamber at the dorsal limbus. A single fragment of tumor tissue was introduced through the incision with a trocar containing a fitted plunger. The tumor fragment was moved to the opposite angle of the anterior chamber by gentle strokes made over the cornea with a spatula. Asepsis was maintained throughout the entire procedure. The eyes were

5. Cheever, F. S., and Morgan, H. R.: Cancer Research **2**:675, 1942.

examined at suitable intervals with a slit lamp and a corneal microscope. The implant was recorded as growing only when it had enlarged at least threefold and had become vascularized. Care must be taken lest corneal vascularization over the implant be mistaken for vascularization of the implant itself. In other experiments the characteristic appearance of enlargement and vascularization of the implant has never been produced by any reaction other than actual growth.

This particular tumor, unlike some other tumors that we have transplanted successfully, consistently produced an ocular inflammatory reaction characterized by a peripheral corneal vascular pannus and uniform clouding of the cornea. The reaction reached its height about the third day and subsided without residua within a week. When growth occurred, it became perceptible between seven and fourteen days. With this tumor, growth was often slight, the anterior chamber being only one quarter or one third filled. In a few instances the growth nearly filled the anterior chamber, and the cornea appeared to be distended, but rupture of the globe never occurred. Involvement of the vitreous body never was observed, and all instances of growth terminated in regression.

EXPERIMENTS

Three separate series of guinea pigs were studied. Each of 17 guinea pigs comprising the first series received a tumor transplant into the anterior chamber of the right eye. In 9 the transplants grew, and in 8 they were absorbed. Later fragments of the same tumor were transplanted into both the right and the left anterior chamber of each of these animals. The tumor grew in both the left and the right eye of only 1 animal. It grew in the left eyes of 2 other animals.

A second series, 6 guinea pigs, was treated in a similar fashion. Growth occurred in the transplants in the right eyes of 4 of the 6 animals. When the tumor was retransplanted into both eyes of each of the 6 animals, growth did not occur in either eye in a single instance.

A third series, 19 guinea pigs, was then used in a like manner. In 8 animals growth occurred in the right eye, and no growth occurred in the remaining 11. Later, when the mouse tumor was transplanted into both eyes, it failed to grow in any of the animals.

Each time that fresh samples of tumor were used their growth potentialities were tested in control animals. Growth occurred in 20 of 31 of these animals. When the tumor was transplanted simultaneously into both eyes of the control animals, growth occurred in both eyes of 8 of 12 animals used.

Three of the guinea pigs which had become refractory to the growth of MT8 in the manner described received subsequently transplants of a human carcinoma which had been maintained through three guinea pig anterior chamber generations. In 2 of the 3 animals the transplantation was successful. In a fourth animal, which had proved resistant to growth of MT8 on two transfers, the human carcinoma was successfully transplanted into the anterior chamber of the eye.

The usual ocular reaction following the primary transplantation of this tumor was absent on the second transplantation in the right eye but was present and only slightly diminished in the left eye. This observation was quite consistent in all of the animals in which retransplantations were made (fig. 3).

The three series can be described in summary as follows: Forty-two guinea pigs received transplants of MT8 in the anterior chamber of the right eye. No growth was observed in 21 of these animals, while in the other 21 growth of the transplanted tumor was observed. In all instances of growth the tumor ultimately

underwent complete regression. After regression was complete, both eyes of each of the 42 animals received new transplants of the same tumor. No growth occurred in either eye of any of the 21 animals which had failed to show growth on the first transplantation. In the 21 in which growth of the initial transplant, in the right eye, had occurred the second transplants, in both eyes, gave rise to growth in both eyes of 1 animal only and in the left eye only of 2 other ani-

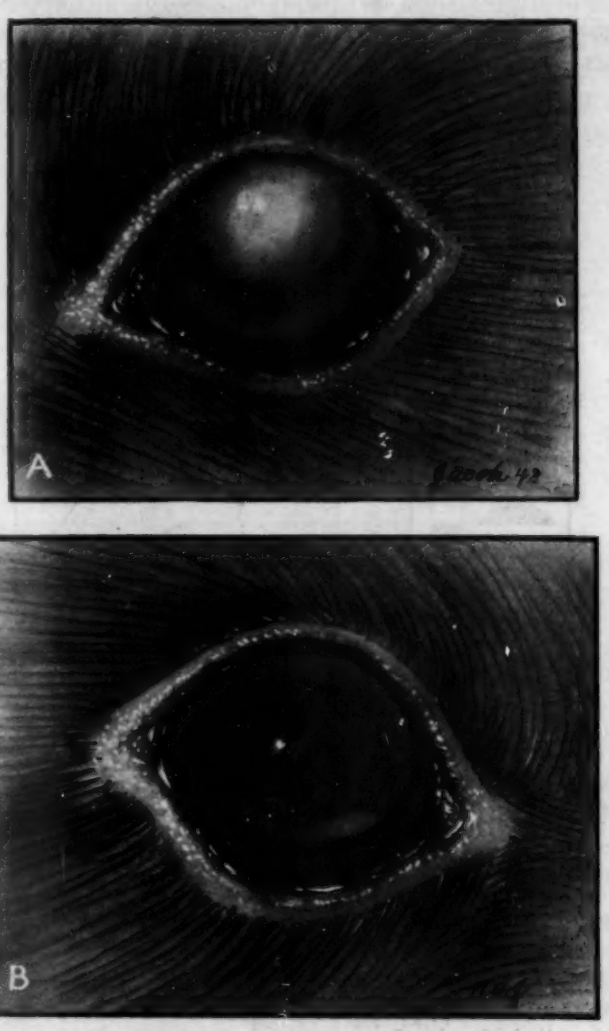

Fig. 3.—*A*, characteristic appearance of the ocular inflammatory reaction evoked by the initial transplant of MT8 in the guinea pig eye as seen on the fourth day after transfer of the tumor fragment. The transplant is obscured by the clouded cornea.

B, characteristic appearance of a guinea pig eye showing a minimal inflammatory reaction to a second transplant of MT8 on the fourth day after transfer of the tumor fragment. The cornea is relatively clear, allowing visualization of the transplant at the inferior angle of the anterior chamber.

mals. The usual ocular inflammatory reaction was absent in the right eyes and diminished in the left eyes on the second transplantations. The time relationships of growth, regression and retransplantations are summarized in figures 4 and 5.

COMMENT

The experiments described tend to confirm the observations of Greene that a state refractory toward intraocular heterologous tumor transplants is produced in response to a growing (or to a regressing?)

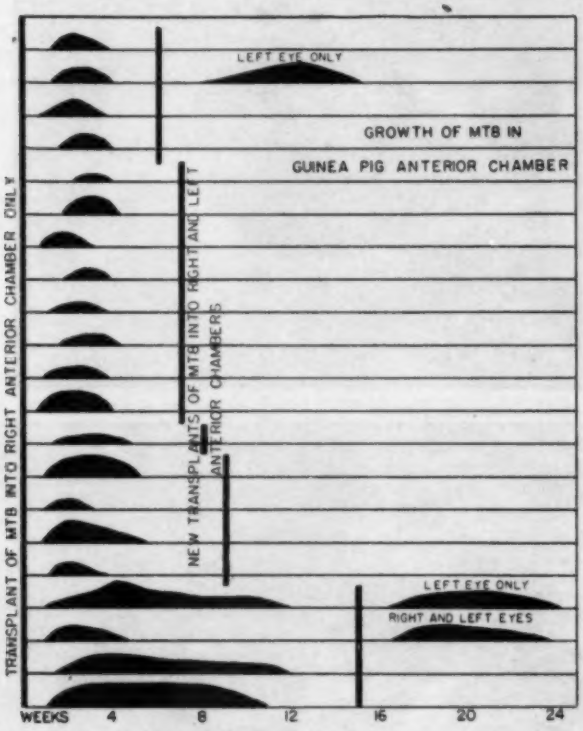

Fig. 4.—Extent and duration of growth of the carcinoma MT8 in initial and secondary transplantations in the guinea pig eye. The blackened area represents the degree of filling of the anterior chamber as well as the duration of growth.

transplant of the same tumor. A monocular transplant confers the refractory state on both eyes. The degree of specificity of this effect has not been studied closely. It was noted, however, that the refractory state applied to the same tumor whether the tumor was carried in C3H or dba strains of mice. Also, 3 of 4 guinea pigs refractory toward growth of MT8 mouse carcinoma were not refractory to growth of intraocular transplants of a human carcinoma that had previously been carried through three generations of anterior chamber transplants.

It is noteworthy that those animals which did not tolerate growth of the first transplants were never subsequently inoculated successfully with the same tumor. (One of these animals tolerated growth of a different tumor.) In other words, half of the experimental animals appeared to have a natural resistance which prevented growth of the transplants. Other animals tolerated growth of the transplants only to a slight and brief extent, while still others behaved as if effective resistance developed slowly and weakly. These differences could be explained by the postulate that the ocular resistance varies among different individuals of the same species. (Uniformity of the tumor samples trans-

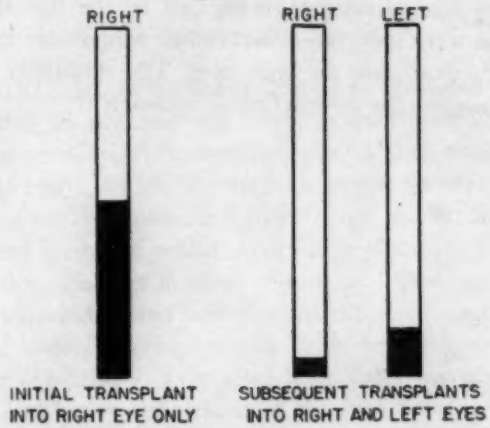

Fig. 5.—Proportions of growth on initial and subsequent transplantations of the carcinoma MT8 in the anterior chamber of the guinea pig eye. The blackened area represents the incidence of successful transfer.

planted was closely checked.) The experimental results indicate that weakly resistant individuals become more effectively resistant after contact with the tumor. Alterations in the reactive state of the eye are further evidenced by the variations observed in the general inflammatory reactions of the part of the cornea and the conjunctiva between initial and subsequent transplants of the same tumor.

The unique receptiveness of the eye to various types of tissue transplants can be described by the generalization that the eye reacts against such transplants relatively weakly or sluggishly. This does not indicate whether the reactions of the eye differ in kind or merely in degree and/or rate from the reactions of other sites. Nevertheless, the relatively protracted and leisurely course of the ocular reactions, coupled with the convenience with which these reactions may be observed through the cornea, affords certain advantages in the study of the fate of heterotransplants.

The individual variations of susceptibility to transplantations and the alterations of susceptibility and of the inflammatory reactions observed after the cornea has been in contact with the tumor provide a basis for suspecting that something in the nature of immune reactions occurs in the case of intraocular heterotransplants. If this is so, immune type reactions could be responsible for the frequently observed phenomenon of regression of temporarily flourishing intraocular heterotransplants.

SUMMARY

After transplants of a mouse tumor, MT8, had temporarily flourished in the anterior chambers of guinea pig eyes and then regressed, a relatively refractory state developed, evidenced by the fact that subsequent transplantations were only rarely successful. Monocular transplants conferred the refractory state on both eyes. The possibility that this may indicate an immune type of reaction is considered.

PARADOXIC EMBOLISM

A Review of the Literature, with Report of a Case in Which This Condition Followed the Administration of "Dicumarol"

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PARADOXIC EMBOLISM is defined as embolism in which the embolus arises from a vein but lodges in a systemic artery instead of in the pulmonary artery. To accomplish this, it must pass through a septal defect in the heart, which is usually a patent foramen ovale.

According to Thompson and Evans,[1] the earliest instance mentioned in the medical literature is that reported by Cohn in 1860. But it was not until 1877 that the first detailed report of a case was given, by Cohnheim. He described recent embolism of the right middle cerebral artery in a case of widely patent foramen ovale with thrombosis of the veins of the lower extremities. Five years later Zahn was able to demonstrate, in a case of extensive thrombosis of the iliac veins, a long embolus the thickness of a pencil passing through a persistent foramen ovale. A similar observation was made by Hauser in 1888. Von Recklinghausen[2] first suggested the term "paradoxic embolism" to describe this condition.

Since the earlier reports, a few isolated cases of paradoxic embolism have been reported, the two largest series consisting of 7 cases each (Thompson and Evans[1]; Ingham[3]). The accompanying table gives a summary of the 41 recorded cases. All of these were verified at autopsy except for that of Porter.[4] In his patient the symptoms and signs were so convincing that a clinical diagnosis could be made with a reasonable degree of certainty, even though the patient recovered. In 33 of the 40 cases confirmed by autopsy, the paradoxic embolism was due to a patent foramen ovale, and in the remainder, to a patent interventricular septum.

From the Departments of Gynecology, Surgery and Internal Medicine of the Lovelace Clinic.

1. Thompson, T., and Evans, W.: Quart. J. Med. **23**:135, 1930.
2. von Recklinghausen, cited by Thompson and Evans.[1]
3. Ingham, D. W.: Am. J. M. Sc. **196**:201, 1938.
4. Porter, A. G.: Lancet **2**:634, 1941.

In about 50 per cent of the cases cerebral embolism was the most conspicuous feature of the disease, while in the rest multiple emboli were present.

The making of a diagnosis of paradoxic embolism during life is usually extremely difficult. In the majority of cases the diagnosis has been made only at autopsy. A notable exception to this is the case reported in 1941 by Porter,[4] already mentioned, in which the patient recovered. According to Porter, such a diagnosis should be seriously considered if in the presence of venous thrombosis followed by pulmonary embolism and recovery there are later signs of infarction in other organs. If the acute attack is preceded by cerebral symptoms, one may consider the diagnosis more positively.

*Cases of Paradoxic Embolism Reported in the Literature**

Author	Year	Cases
Cohn, B.: Klinik des embolischen Gefasskrankheiten, Berlin, A. Hirschwald, 1860	1860	1
Cohnheim, J.: Vorlesungen über allgemeine Pathologie, Berlin, A. Hirschwald, 1877, vol. 1, p. 131	1877	1
Louis, cited by Ballet, G.: Arch. gén. de méd. 5:659, 1880	?	1
Zahn: Rev. méd. de la Suisse 1:227, 1881	1881	1
Rostan: Thesis, Geneva, 1884	1884	1
Hauser: München. med. Wchnschr. 35:583, 1888	1888	3
Ohm: Ztschr. f. klin. Med. 61:374, 1907	1907	1
Hensel, R.: Deutsche med. Wchnschr. 47:625, 1921	1921	1
Beattie: Internat. A. M. Museums Bull. 11:64, 1925	1925	2
Versé: Verhandl. d. deutsch. path. Gesellsch. 13:215, 1930	1930	2
Bernard: Quart. J. Med. 23:305, 1930	1930	1
Thompson and Evans[1]	1930	7
French: Arch. Path. 11:383, 1931	1931	1
Taylor: Arch. Path. 16:901, 1933	1933	1
Armand-Delille and Lesobre: Bull. Soc. de pédiat. de Paris 33:274, 1935	1935	1
Hirschboeck, F. J.: Am. J. M. Sc. 189:236, 1935	1935	1
Koritschoner, R.: J.A.M.A. 106:1269, 1936	1936	1
Neely: Nebraska M. J. 21:61, 1936	1936	1
Jones, R.: Brit. M. J. 2:225, 1936	1936	1
Ingham[3]	1936	7
Hanna, R.: Am. J. Dis. Child. 62:555, 1941	1941	1
Porter[4]	1941	1
Vintrup, B.: Nord. med. (Hospitalstid.) 10:1839, 1941	1941	2
Birch, C. A.: Brit. M. J. 2:727, 1945	1945	1

*All but the case of Porter (1941) were confirmed by postmortem examination.

The following case is reported not only because of the interest aroused by the rarity of such a lesion but also because, so far as can be determined, it is the first case in which such a complication has followed the use of an anticoagulant drug.

REPORT OF CASE

A 50 year old white woman, married, first seen on July 29, 1947, complained that there had been profuse vaginal bleeding for the preceding three days, pelvic discomfort for six months and large, painful varicosities of both lower extremities which had become gradually worse during the preceding few years.

The family history was noncontributory. The past history was essentially irrelative except that for many years the patient had suffered recurrent attacks of pain in the right upper quadrant of the abdomen, accompanied by nausea and vomiting, concerning which a diagnosis of disease of the gallbladder had been

made elsewhere. She had had seven uneventful term pregnancies. Her menstrual function had been normal throughout her reproductive life, and she stated that her last normal menstrual period had occurred about one year before her present illness. Specifically, there was no past history suggestive of cardiovascular disease.

She was a well developed, moderately obese white woman about 50 years old, who did not appear to be in acute distress. The skin and the mucous membranes were normal. The heart and the lungs were entirely normal, and the blood pressure 140 systolic and 90 diastolic. Except for the palpation of a hard abdominal mass arising from the lower part of the pelvis and extending 12 cm. above the symphysis, abdominal examination showed no abnormality. The liver was not enlarged, and there was no tenderness in the region of the gallbladder. Pelvic examination confirmed the presence of a uterine tumor of the size described; the adnexa could not be palpated because of obesity. The cervix was patulous and bore old, healed lacerations. The patient was bleeding moderately from the uterine cavity, and the vagina was filled with freshly clotted blood. There were extensive varicosities of both internal saphenous systems.

Urinalysis gave normal results. The red blood cell count was 4,300,000; the hemoglobin content, 86 per cent. The white cell count was 7,800, with a normal differential count. The Kahn test revealed no syphilis.

A preoperative diagnosis of leiomyoma and varicose veins was made. The following day ligation of the saphenous veins was done by one of us (R.C.D.), followed immediately by total hysterectomy, bilateral salpingo-oophorectomy and appendectomy (R.L.Y.). Findings at the time of operation confirmed the presence of a uterine tumor measuring 13 by 10 by 8 cm. A diagnosis of adenomyosis uteri, chronic salpingitis and chronic cystic cervicitis was made. Palpation of the gallbladder did not reveal stones, nor were abnormalities of other abdominal organs found. Bleeding at the time of operation was not excessive or difficult to control, a liberal estimate of 150 cc. total blood loss being made.

Immediately after the operation she was given intravenously 1,000 cc. of an isotonic sodium chloride solution containing 5 per cent dextrose. The postoperative course was uneventful until twenty-four hours after the operation, when she began to bleed profusely from all the incisions—the abdominal, the vaginal and both of the subinguinal incisions—and rapidly entered into profound shock. The blood pressure and the radial pulse were unobtainable; the apical pulse rate was 160 per minute. One hour after the onset of bleeding, the red blood cell count was 2,810,000; the hemoglobin content, 7.8 Gm. (50 per cent); the platelet count 281,000; the coagulation time, 4.5 minutes; the bleeding time, 1 minute; the prothrombin time (Quick), 27 minutes, or less than 1 per cent.

During the next six hours she received 250 cc. of blood plasma, 250 cc. of isotonic sodium chloride solution and 1,500 cc. of freshly citrated whole blood. In addition, 60 mg. of vitamin K was given intravenously every four hours until the prothrombin time returned to normal. Bleeding from the wounds gradually decreased but did not cease completely until twenty-four hours after its onset. The blood pressure first returned to normal eight hours after the onset of the bleeding.

Twenty-four hours later the prothrombin content was 100 per cent, the red blood cell count 2,900,000 and the hemoglobin content 9.0 Gm. The skin for about 8 cm. around the abdominal wound was intensely ecchymotic, and the arms presented ecchymotic areas wherever hypodermic injections had been given. Additional whole blood was given during the next few days until the hemoglobin was 11.0 Gm., and her condition gradually improved. Liver function tests were made to determine the cause of the great increase of prothrombin time. The

sulfobromophthalein sodium test revealed 6.5 per cent retention in forty-five minutes. Roentgenograms of the gallbladder revealed a nonfunctioning organ without evidence of stones.

Because of a persistent low grade daily fever, penicillin was given. An infected hematoma soon developed in the abdominal wound and was evacuated on the fourteenth postoperative day, yielding about 200 cc. of old blood. This continued to drain saniopurulent material throughout the postoperative course.

Four weeks after the episode of bleeding, when her condition was apparently good, the patient confided that prior to undergoing surgical treatment she had consulted an osteopathic physician, who had given her a week's supply of small white capsules with instructions to take one three times a day "to keep the blood in her varicose veins from clotting." On further investigation the capsules proved to contain 50 mg. of "dicumarol" (3,3'-methylenebis [4-hydroxycoumarin]) each. It was estimated that she had taken a total of 950 mg. of "dicumarol" during the week preceding the operation. During the administration of this drug, prothrombin times were not determined, and the patient remained ambulatory in another town, having been advised to return for further examination in a week.

Five weeks after the operation she suddenly complained of pain in the left side of her chest. Examination revealed a pleural friction rub over the lower lobe of the left lung posteriorly, and a roentgenogram showed, near the cardiac apex, an area of consolidation which measured about 2 cm. in diameter. A final differentiation between pulmonary embolism and pneumonia was not made, but further anticoagulant drugs were not given. Low grade fever continued despite antibiotic drugs, but the patient had no further complaints.

One week later she suddenly became cyanotic and orthopneic and complained of inability to move the right foot and leg. Shortly thereafter she complained of weakness of the left hand. Both lower extremities were pale, cold and clammy. There was no limitation of voluntary motion of the left leg, though the patient was unable to move the right leg. The grip of the left hand was definitely weaker than that of the right. A diagnosis of saddle embolus was entertained, with possibly a cerebral accident, but because of the infection of the abdominal wall, embolectomy was not attempted. The patient was treated with morphine, papaverine and oxygen. Twelve hours later the right leg became intensely painful, cold and cyanotic. A diagnosis of embolism of the right iliac artery was made, and sympathetic nerve block was undertaken. The patient died while this procedure was being performed.

Autopsy (six hours after death, by T. R. Moran).—A superficial abscess of the fat was present in the operative scar. The liver was not enlarged. The right femoral and common iliac arteries contained an antemortum clot 15 cm. long. The right hypogastric vein was filled with a thrombus. The inferior vena cava contained a hard, dry clot, which extended throughout its length but did not completely fill the lumen. The aorta contained an embolus, which extended from the heart itself throughout the aortic arch. A patent foramen ovale was present which measured 9 mm. in diameter. The semilunar valve was held open by an embolus; the short rounded end of this protruded into the left auricle, while the proximal longer end extended into the right auricle. Both pulmonary arteries contained massive, firm, dry emboli. Both lungs revealed patchy areas of infarction. Other organs were grossly normal. The brain was not examined.

COMMENT

Although patency of the foramen ovale is one of the commonest of all congenital abnormalities, being found in from 20 to 35 per cent of

all autopsies, there are several factors which contribute to the rarity of paradoxic embolism. The first of these is the size of the septal defect. Thompson and Evans[1] found a patent foramen ovale in 386 of 1,100 autopsies (35.1 per cent). In 319 cases (29 per cent) the opening was only large enough to admit a small probe. In only 67 cases (6 per cent) could an ordinary lead pencil be passed through the foramen.

The second factor is the pressure relationship between the auricles. In the majority of cases of patent foramen ovale, the opening on the side of the left auricle is guarded by a valvelike fold of endocardium. Since the pressure in the left auricle is normally higher than that in the right, auricular contraction will insure closure of the defect, thus effecting physiologic competence.

According to Wittig,[5] however, 50 per cent of all the cases of paradoxic embolism are preceded by pulmonary embolism. This results in a rise of pressure in the right auricle and a decrease in the left, thus allowing blood to flow from right to left through the patent foramen. Before this can occur, however, it is necessary for at least one third of the pulmonary circulation to be occluded. Since occlusion of 50 per cent or more of the pulmonary circulation causes immediate death, it is obvious that many of the patients will die before paradoxic embolism has had time to develop. Furthermore, if a nonfatal infarct has been formed of sufficient size to increase the pressure in the right auricle—thus setting the stage for the development of paradoxic embolism—it is still necessary for a second embolus to reach the right auricle from a peripheral vein. If, as is often the case, the embolus represents a cast of the vessel in which it originates, it is obvious that the foramen ovale must be of rather large size to permit its passage.

In the 50 per cent of the cases in which paradoxic embolism is not associated with pulmonary embolism, the explanation is more difficult. One suggestion is that minute bacterial emboli are able to pass through small defects and lodge in the peripheral arteries.

In our own case a small pulmonary embolus had occurred at least one week before death. While this embolus was hardly of sufficient size to elevate the pressure of blood within the right auricle, the second and more massive pulmonary embolus which was found at autopsy undoubtedly preceded the development of the paradoxic embolus. The septal defect measured 9 mm. in its greatest diameter; once pressure relationships between the auricles were reversed, paradoxic embolism was the almost inevitable sequel.

As in 50 per cent of the recorded cases, clinical signs of cerebral embolism were evident shortly before death. Although examination of the

5. Wittig, M.: Ztschr. f. Kreislaufforsch. 19:505, 1927.

brain was not permitted, it can reasonably be assumed that cerebral embolism was present.

As has already been mentioned, this is the first recorded case in which paradoxic embolism has followed the use of an anticoagulant drug. Whether or not its administration was responsible for the death of this patient can, of course, only be postulated. While the danger of thromboembolic disease is always present after a pelvic operation, certain conditions known to predispose to its development were either caused or aggravated by the administration of "dicumarol." Notable among these were the long-continued venous stasis resulting from the profuse postoperative bleeding and the massive, infected hematoma of the abdominal wall.

Despite repeated warnings in the medical literature and the explicit instructions which accompany the product, the improperly controlled use of "dicumarol" continues[6] In 1943, Link,[7] the synthesist of this drug, commented that "the briefest meditation on the strictly theoretical aspects of the clotting phenomenon leaves us with the appalling feeling that tampering with coagulability of the blood . . . is a hazardous business." It is hoped that, as more and more cases of "dicumarol" poisoning are reported, physicians who undertake the responsibility of administering "dicumarol" will listen to Link's warning.

SUMMARY

Forty-one cases of paradoxic embolism have been collected from the literature. These are reviewed, and an additional case, the first to be complicated by "dicumarol" poisoning, is reported.

Factors predisposing to the occurrence of paradoxic embolism are discussed, and the probable mechanism of development is outlined.

The possible influence of the administration of "dicumarol" on the development of paradoxic embolism is discussed, and warning is again made against the improperly controlled use of this drug.

6. Draper, A. J.: J.A.M.A. **136**:171, 1948.

7. Link, K. P.: Anticoagulant From Spoiled Sweet Clover Hay, in Harvey Lectures, 1943-1944, Baltimore, Williams & Wilkins Company, 1944, p. 162.

HEREDITARY RENAL DISEASE AND AMYLOIDOSIS IN MICE

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MOST strain A mice in which tumors of the mammary glands do not develop die of a degenerative disease of the kidney commonly referred to as nephritis. The onset of the condition occurs in approximately 100 per cent of the animals by 12 months of age, and few live much beyond 18 months.

In 1938, Andervont[1] noted the common occurrence of nephritis in this strain, and later Gorer[2] referred to the condition as cystic disease of the kidneys and described it as a special type of hydronephrosis in which obstruction of the tubules was brought about by focal necrosis occurring at the tip of the renal papilla. A more comprehensive description of the pathologic aspects of the condition has been given by Dunn,[3] who related the renal disorder to amyloidosis. She described the course of the disease as beginning with deposition of amyloid between the tubules of the papilla, followed by obstruction of the tubules, which results in destructive changes that radiate to the surface of the kidney.

While the condition has been emphasized in regard to strain A, it also occurs in strain Y. Approximately 100 per cent of the mice of this strain show the condition as early as those of strain A do. In certain other strains, however, the incidence is low. In the study reported herein a low incidence was recorded for strain L. Gorer reported that the disease had been observed in only a few strain C_{57} black mice and in those at an advanced age. He did not find it in his series of strain CBA. Dunn recorded that but 1 of 150 strain C_3H mice examined was affected.

These strain differences indicate a genetic background for the condition and suggest that hybridization studies would be of interest. F_2 and back-cross ratios could be expected to give some indication of the num-

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From the National Cancer Institute, National Institute of Health, United States Public Health Service.

1. Andervont, H. B.: Pub. Health Rep. **53**:232, 1938.
2. Gorer, P. A.: J. Path. & Bact. **50**:25, 1940.
3. Dunn, T. B.: J. Nat. Cancer Inst. **5**:17, 1944.

*Occurrence of Nephritis and Amyloidosis in Strains A, L and Y and Their Hybrids**

Type of Mice	Age in Months																	Total	Per Cent
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Strain A mice				1	3	19	12	4	4	7	35							85	
Number with nephritis				1	2	14	11	4	4	7	35							78	91.8
Number with amyloidosis				1	3	17	10	4	4	7	35							81	95.3
Number with both				1	2	13	9	4	4	7	35							75	88.2
Strain L mice			3(1)	1(0)	1(0)	1	2	4	14(12)	7	83(77)	3	4(3)	10	6	3	25	167(154)	
Number with nephritis			2(0)	1(0)	1(0)	0	0	1	1(0)	0	4(3)	1	1(0)	1	1	1	4	19(12)	7.8
Number with amyloidosis			2(0)	1(0)	1(0)	0	0	0	2(1)	0	6(5)	2	3(2)	1	2	1	10	31(24)	15.6
Number with both			2(0)	1(0)	1(0)	0	0	0	1(0)	0	4(3)	1	1(0)	0	1	1	2	15(8)	5.2
LAF_1 hybrids										1	42(41)	1	2	4(3)	3	7	77(73)	137(131)	
Number with nephritis										0	1(1)	0	0	1(0)	0	0	5(1)	7(2)	1.5
Number with amyloidosis										1	1(0)	0	0	2(1)	1	5	31(27)	41(35)	26.7
Number with both										0	0(0)	0	0	1(0)	0	0	5(1)	6(1)	0.8
ALF_1 hybrids		1			3	1		2		1	35	1	3	2	2	3	31(30)	85(84)	
Number with nephritis		0			0	0		0		0	0	0	2	0	0	1	3(2)	6(5)	6.0
Number with amyloidosis		0			0	0		0		0	3	0	1	2	0	2	28(27)	36(35)	41.7
Number with both		0			0	0		0		0	0	0	1	0	0	1	3(2)	5(4)	4.8
F_1 hybrids (combined)		1			3	1		2		2	77(76)	2	5	6(5)	5	10	108(103)	222(215)	
Number with nephritis		0			0	0		0		0	1	0	2	1(0)	0	1	8(3)	13(7)	3.3
Number with amyloidosis		0			0	0		0		1	4(3)	0	1	4(3)	1	7	59(54)	77(70)	32.6
Number with both		0			0	0		0		0	0	0	1	1(0)	0	1	8(3)	11(5)	2.3
LAF_2 hybrids			1	2(1)	6(3)	5(0)	5(0)	1	3(2)	2	45(42)	2(1)	2	6(5)	6(5)	1	18(17)	105(83)	
Number with nephritis			0	1(0)	2(0)	2(0)	3(0)	1	2(1)	2	23(21)	1	1	2(1)	4(3)	0	4(3)	48(34)	41.0
Number with amyloidosis			0	1(0)	4(1)	2(0)	5(0)	0	2(1)	2	28(25)	1	1	4(3)	5(4)	0	10(9)	65(47)	56.6
Number with both			0	1(0)	2(0)	2(0)	3(0)	0	2(1)	2	23(21)	1	1	2(1)	4(3)	0	3(2)	46(32)	38.6
ALF_2 hybrids	1	1		1	2(0)		1	1(0)	8	1(0)	44(40)	2	4(3)		2(1)	2	17(16)	87(76)	
Number with nephritis	0	0		0	1(0)		1	1(0)	4	1(0)	6(3)	0	2(1)		1(0)	1	3(2)	21(12)	15.8
Number with amyloidosis	0	0		0	1(0)		1	1(0)	5	1(0)	11(8)	1	2(1)		2(1)	1	12(11)	38(29)	38.2
Number with both	0	0		0	1(0)		1	1(0)	4	1(0)	5(3)	0	2(1)		1(0)	0	3(2)	19(11)	14.5
F_2 hybrids (combined)	1	1	1	3(2)	8(3)	5(0)	6(1)	2(1)	11(10)	3(2)	89(82)	4(3)	6(5)	6(5)	8(6)	3	35(33)	192(159)	
Number with nephritis	0	0	0	1(0)	3(0)	2(0)	4(1)	2(1)	6(5)	3(2)	29(24)	1	3(2)	2(1)	5(3)	1	7(5)	69(46)	28.9
Number with amyloidosis	0	0	0	1(0)	5(1)	2(0)	6(1)	1(0)	7(6)	3(2)	39(33)	2	3(2)	4(3)	7(5)	1	22(20)	103(76)	47.8
Number with both	0	0	0	1(0)	3(0)	2(0)	4(1)	1(0)	6(5)	3(2)	29(24)	1	3(2)	2(1)	5(3)	0	6(4)	66(43)	27.0

Type of Mice	Age in Months																	Total	Per Cent
	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
A back-cross hybrids	1	1	0	2	3(2)	1(0)	0	2(1)	2	5(4)	56(54)	1	4(3)	1(0)	6	1	22(19)	108(97)	
Number with nephritis	0	0	0	0	0(0)	0	0	2(1)	1	5(4)	33(32)	1	4(3)	1(0)	5	1	3(3)	56(51)	52.6
Number with amyloidosis	0	0	0	1	1(0)	1(0)	0	2(1)	1	5(4)	36(35)	1	3(2)	1(0)	5	1	14(11)	72(62)	63.9
Number with both	0	0	0	0	0	0	0	2(1)	1	5(4)	32(31)	1	3(2)	1(0)	4	1	3(3)	52(48)	49.5
L back-cross hybrids		2		4(2)	6		2	6(4)	1	1	27(19)		5(3)	6(4)	1(0)	2	13	76(69)	
Number with nephritis		0		0	0		0	0	0	1	3(0)		1	1(0)	1(0)	0	1	8(3)	4.3
Number with amyloidosis		0		0	1		2	0	0	0	7(2)		0	2(1)	1(0)	2	10	25(18)	23.7
Number with both		0		0	0		0	0	0	0	3(0)		0	1(0)	1(0)	0	1	6(1)	1.4
Strain Y mice	1				3													4	
Number with nephritis	1				3													4	100
Number with amyloidosis	0				3													3	75
Number with both	0				3													3	75
AYF_1 hybrids								90										90	
Number with nephritis								90										90	100
Number with amyloidosis								90										90	100
Number with both								90										90	100

*Numbers of mice in groups after mice with secondary amyloidosis have been excluded are given in parentheses. The percentages relate to the restricted groups.

ber of genetic factors involved, and the segregation of amyloidosis in relation to that of the renal disease could be expected to confirm or deny their relationship and, furthermore, would indicate which condition was more directly the result of the genic action. This paper reports the results of such hybridization studies.

PROCEDURE

The work was concerned principally with an analysis of the difference between the high nephritis strain A and the low nephritis strain L (referred to as "strain C_{57} leaden" by Law[4]) but observations on high nephritis strain Y and the first generation hybrids between strain Y and strain A were also recorded. All three are highly inbred strains and are of the colonies maintained at the National Cancer Institute. All were derived from the stocks of the Roscoe B. Jackson Memorial Laboratory. The strain A and strain L stocks were obtained in 1938 and the strain Y stock in 1936.

Reciprocal matings were made between the two parent strains A and L for producing the ALF_1 hybrids with strain A mothers and LAF_1 hybrids with strain L mothers. These reciprocal F_1 hybrids were in turn mated inter se to produce ALF_2 and LAF_2 hybrids, and also were mated to the parent strain A to produce A back-cross hybrids and to the parent strain L for L back-cross hybrids. Mice of the two parent strains and of the hybrid groups were segregated as to sex and maintained in wooden cages with 8 mice to the cage except during the time they were in the breeding cages. Then 4 females were mated to 1 male and when pregnant were isolated in separate cages for rearing their litters. All groups were fed Purina dog chow and were given a constant supply of tap water.

At the outset it was planned to kill and examine sample groups at 18 and 24 months of age in order to tabulate the number with amyloidosis and the number with nephritis at these age periods. Some of the animals, however, were found moribund or dead and had to be examined at other age levels. None of the strain A mice were kept beyond 18 months, because at this age their mortality was high. None of the other groups were kept beyond 24 months.

The kidneys of all mice were fixed and sectioned so as to include the papilla to ascertain the presence or the absence of degeneration at this site. After establishing the fact that the amyloidosis was systemic, the routine diagnosis was made on observation of sections of only the spleen and the duodenum. These organs were chosen because the interstitial tissue of Brunner's glands and the spleen have been found to be the two locations in which amyloid is most likely to occur in the mouse. The tissues were fixed in Tellyesniczky's fluid (70 per cent ethyl alcohol, 20 parts; formaldehyde solution U.S.P., 2 parts; glacial acetic acid, 1 part) and were stained with hematoxylin and eosin. The material considered as amyloid had the same localization as that which Dunn[3] described when working in this laboratory with the strain A mice, and has been identified by Turnbull,[5] after using special straining procedures, as mouse amyloid.

To obtain data on a cross between two strains both of which had a high occurrence of amyloidosis and nephritis, sections were made of the kidneys, duodenums and spleens of 4 strain Y males and a sample group of 90 F_1 hybrids resulting from crossing these strain Y males to strain A females. Three of the

4. Law, L. W.: J. Hered., to be published.
5. Turnbull, H. M.: J. Path. & Bact. 57:18, 1945.

strain Y males were killed and examined at 12 months of age and 1 at 8 months, and all of the AYF_1 hybrids were killed and examined at 15 months of age. These animals were a part of an experiment for analyzing the relationship of the lethal yellow gene and spontaneous pulmonary tumors, but had been reared under conditions identical with those described for the other groups.

RESULTS

The rates at which amyloidosis and nephritis occurred in the inbred lines and in the various hybrid groups are recorded in the table. Aside from listing the total number, the number with nephritis and the number with amyloidosis, there is listed also the number with both conditions. From these figures one can readily determine the number with nephritis even though amyloid was not observed, or the number with amyloidosis and not nephritis.

Unfortunately, the picture was somewhat confused by the fact that in certain of the animals secondary amyloidosis was noted—distinguished from the primary condition by the presence of some other chronic disease. For the most part these were animals which, in consequence of being infested by the small mite Myobia musculi, had had severe dermatitis for a considerable period. Persistent scratching of the dorsal region of the head had resulted in extensive destruction of tissue; in some mice even the ears and the eyes were eroded. Lymph nodes, particularly those of the cervical region, were often greatly enlarged. In a few other animals large abscesses or other chronic infections suggested the possibility that the amyloidosis could be secondary. Generally in these animals the amyloidosis had a slightly different manifestation in that infiltration occurred earlier and to a greater degree in the spleen than in the duodenum. Nephritis occurred in the mice showing secondary amyloidosis just as in those that showed primary amyloidosis.

For the genetic analysis we eliminated the data concerning mice in which examination showed chronic diseases that could have caused amyloidosis, and determined incidence of amyloidosis and nephritis and segregation ratios only on the restricted data. Both the original and the restricted data are included in the table.

No sex difference was found in the incidence of either amyloidosis or nephritis in any group, and therefore the sexes are not segregated in the final tabulation. In all groups, however, the number of males and and the number of females were approximately equal.

The data concerning the strain A mice confirm previous observations that most animals of this strain show both amyloidosis and nephritis at 12 months of age or older. All of the group killed and examined at 18 months showed both conditions, as did all those in the 15, 16 and 17 month age groups on which autopsies were made because they were moribund or had died. Approximately 90 per cent of all age groups (11

to 18 months) had both amyloidosis and nephritis. Of the 12, 13 and 14 month groups, a few had nephritis without amyloid being apparent in the spleen or the duodenum. In a slightly greater number amyloid was found in the spleen and the duodenum without apparent degenerative changes in the kidney.

Comparatively few of the strain L mice had either amyloidosis or nephritis, and this number was decreased by excluding those which had apparent secondary amyloidosis, which occurred more often among the animals that had to be examined at the earlier ages. Only 3 of the 77 of the restricted group killed at 18 months showed the changes of the kidney, and in each of these 3 amyloid was also found. In 2 animals amyloid was found without the onset of renal changes. With advancing age the incidence of both conditions appeared to increase slightly, with possibly a preponderance of amyloidosis.

The first generation hybrids resulting from crossing strain A with strain L were comparable to the parent strain L. The combined data for the reciprocal groups show that only 1 animal of the 76 of the restricted lot killed and examined at 18 months had nephritis and only 3 had amyloidosis. Again as the age increased the incidence of each condition increased slightly, with the number of mice showing amyloidosis exceeding the number showing nephritis.

To test for a maternal influence reciprocal hybrids were produced. In the comparison of these two F_1 groups a difference was suggested, those with strain A mothers having a higher incidence of both amyloidosis and nephritis than those with strain L mothers. Statistically the differences of the data for the F_1 groups were borderline in significance: For amyloidosis $X^2=5.208$; $P=0.02$; for nephritis $X^2=3.18$; $P=0.07$. This difference, however, was not borne out in the reciprocal F_2 hybrids; hence the difference in the F_1 generation may not have been a real difference or, if real, not due to maternal factors that were passed on to the next generation.

In the F_2 generation evidence of segregation appeared. The LAF_2 and ALF_2 groups combined totaled 192, of which 159 were classified in the restricted group. Of these 159, 46, or 28.9 per cent, had nephritis. Considerably more, 76, or 47.8 per cent, had amyloidosis, and 43 of the 46 with nephritis showed amyloidosis. There was an increase in amyloidosis with increase in age, but in this group an increase in nephritis was not evident beyond 18 months.

In this generation there again appeared to be a difference in the two reciprocal groups, the incidence of both nephritis and amyloidosis being higher in the LAF_2 hybrids than in the ALF_2. Furthermore, this difference was statistically significant; for nephritis $X^2=12.23$; $P=<0.01$, and for amyloidosis $X^2=5.42$; $P<0.02$. The variation, however, was in the

direction opposite to that in the corresponding reciprocal F_1 groups, which makes the difference difficult to explain, if it is real.

In the generation that resulted from back-crossing the F_1 to the parent strain A, segregation of genetic factors was also apparent. In the restricted group there was a total of 97 of these back-cross animals of all age groups combined, and, of these, 51, or 52.6 per cent, had nephritis. Forty-eight of those with nephritis also showed amyloidosis, and an additional 14 showed amyloidosis without nephritis.

The results for the hybrids produced by back-crossing the F_1 to the parent strain L were comparable to those for the strain L and the F_1 generation in that the incidence of nephritis was low. Only 3 of the 69 animals of the restricted group showed nephritis, although considerably more, 18 of the 69, had amyloidosis.

The strain Y animals included in the data here were characteristic for the strain. All 4 had nephritis, although in 1 killed at 8 months amyloid was not found in the organs sectioned. Extensive amyloid was found in the 3 killed at 12 months.

Of the sample group of 90 F_1 hybrids produced by mating strain A females to strain Y males examined at 15 months of age, all showed extensive amyloidosis, with the degenerative changes in the kidneys classified as nephritis.

COMMENT

The data presented herein indicate that we are dealing with two closely associated degenerative conditions. Whether or not these conditions appear in an animal may be determined both by differences in nongenetic and by differences in genetic factors.

Strain differences are clearly indicated. One can expect practically all strain A mice that reach 15 months of age to show both conditions. While the number of animals of strain Y included in the work reported here is small, the experience we have had with the strain has shown in general that all of this strain would likewise show both conditions and at an age comparable to that noted for strain A. In contrast very few mice of strain L can be expected to present either condition. Such strain differences suggest the influence of genetic factors.

More conclusive evidence, however, of these genetic differences was found in the F_2 and back-cross generations that gave incidence rates indicating the results of the segregation of genetic factors. When the two high nephritis lines A and Y were mated, amyloidosis and nephritis developed in all the AYF_1 hybrids, but when a high nephritis line A was mated to a low nephritis line L, the ALF_1 and LAF_1 hybrids displayed the same low incidence that was observed in the low parent line, suggesting a recessive genetic factor or factors. By mating these ALF_1 and LAF_1 hybrids inter se, however, the incidence was raised as one would

expect, owing to the segregation of these genetic factors. Furthermore, when the F_1 animals were back-crossed to strain A, the incidence observed the A back-cross generation was higher than that in the F_1, but the incidence in the L back-cross generation remained low, comparable to that in the L and the F_1. Such a picture is conclusive evidence of segregating genetic factors.

The influence of nongenetic factors was also clearly indicated. Most prominent of these was chronic inflammation which resulted in secondary amyloidosis with tubular degeneration in the kidney, as mentioned earlier. Most of the animals in which this was a factor, however, could be identified by the presence of the chronic dermatitis or of other chronic inflammation together with the excess of amyloidosis of the spleen characteristic of the secondary type. Secondary amyloidosis was not apparent in the strain A groups, but none of this strain was kept beyond 18 months of age and the primary amyloidosis had developed in most of them at an even much earlier age. The secondary type was also not a factor in the few strain Y animals which were killed at a comparatively early age or in the AYF_1 hybrids killed at 15 months. It was, however, a factor in the strain L groups and the A by L hybrid groups; thus it necessitated the elimination of the data concerning these animals before further analysis of the data bearing on the causative factor of the primary condition was attempted.

The influence of additional and unknown nongenetic factors was evidenced in the cases of amyloidosis and nephritis remaining after the elimination of the identified cases of secondary amyloidosis in the strain L and LAF_1 groups. Individual variation in these groups could not be attributed to genetic variations, for these groups were genetically uniform and therefore individual differences would have to be attributed to variation in nongenetic factors.

The observation that amyloidosis occurred in connection with the nephritis in these cases substantiates Dunn's conclusion that the two conditions are related in that the tubular degeneration of the kidney results from obstruction due to the amyloid present in the intertubular connective tissue of the papilla. In most of the cases of nephritis amyloid was found in the spleen or the duodenum or both. In the few remaining cases it could have occurred in the kidney but was not apparent, owing to further degeneration of the papilla. More frequently amyloidosis was observed without renal changes, particularly in the older groups, indicating that the amyloid appeared earlier.

It is particularly important to emphasize the association of the two conditions in the hybrid groups where segregation of genetic factors is evident. This can be expected when two characters are linked or when both are due to the same genetic factor or factors. In this case, how-

ever, there is no evidence of linkage of genes for the two conditions but rather there is evidence that both result from the same gene.

The data indicate that but one pair of genetic factors is involved, with the gene for nephritis and amyloidosis recessive to the normal allele. The ratio of animals with nephritis to those without nephritis in the F_2 generation does not differ significantly from the 1 to 3 ratio expected with a single recessive factor. By comparing the observed with the expected incidence for the combined restricted groups one obtains an X^2 value of 0.787, with P between 30 and 50 per cent. Furthermore, the ratio in the strain A back-cross generation is not significantly different from the 1 to 1 ratio of a single recessive factor ($X^2=0.129$; P is between 50 and 70 per cent), and the incidence observed in the strain L back-cross is comparable to that in the parent strain L. Each group, however, has an excess of cases of amyloidosis, but this may be accounted for by nongenetic factors. The greater excess occurred in the older animals, an observation suggesting that in some of these cases the amyloidosis might have been secondary although the causative chronic disease was not identified.

While the data suggest a single recessive factor, a final conclusion cannot be drawn from these ratios alone. Ratios simulating those of single factor inheritance can occur in multiple factor inheritance where the presence or the absence of the character is determined by whether or not the combined effects of the genetic and nongenetic factors exceed a physiologic threshold. This condition was well demonstrated by Wright[6] for polydactyly in the guinea pig and has been found also for the inheritance of pulmonary tumors in mice (Heston[7]). The possibility of this type of inheritance thus emphasizes the necessity of breeding tests of the back-cross or F_2 segregants to confirm single factor inheritance.

Since the renal changes evidently result from the deposition of amyloid, it is in the causation of the amyloidosis that one would look for the more direct effect of the genic action. In the light of the lack of knowledge of the metabolic disturbances resulting in the formation of amyloid, however, one hesitates to postulate a possible mechanism through which genetic variation may influence its occurrence. Aside from the secondary amyloidosis that accompanies some chronic diseases, experimental amyloidosis can be produced in mice by the injection of sodium caseinate as shown by Kuczynski[8] and used by a number of later workers. In contrast it has been found[9] that the primary amyloidosis

6. Wright, S.: Genetics **19**:537, 1934.

7. Heston, W. E.: J. Nat. Cancer Inst. **3**:69 and 79, 1942.

8. Kuczynski, M. H.: Virchows Arch. f. path. Anat. **239**:185, 1922.

9. Heston, W. E.; Larsen, C. D., and Deringer, M. K.: J. Nat. Cancer Inst. 6:41, 1945.

characteristic of strain A is inhibited when mice of this strain are fed certain diets restricted in protein, particularly in protein yielding cystine. Amyloid is known to contain protein and probably arises through some disturbance of protein metabolism. Since much genic action becomes manifest through enzymatic action, it seems logical that the amyloid described here could result from a recessive gene effecting an enzymatic deficiency in protein metabolism.

In discussing this renal condition in relation to those in man, Dunn suggested that the term "papillonephritis" be applied, since it emphasizes the involvement of the papilla and does not suggest an analogy to any clearly differentiated type of nephritis in man. The relationship of the amyloidosis and the renal disorder, however, lays emphasis on the amyloidosis, since it is in the formation of the amyloid that one should seek the causative factors. Primary systemic amyloidosis occurs less frequently in man than secondary amyloidosis, but, as Iverson and Morrison[10] have recently pointed out from their studies on cases of the primary condition, little has been indicated regarding the cause of the condition. The demonstration of the genetic factor causing amyloidosis in the mouse suggests that investigations directed toward discovering a genetic factor in primary systemic amyloidosis in man might be fruitful.

SUMMARY

These studies on the nephritis and amyloidosis that occur in mice of the high nephritis strains A and Y and the low nephritis strain L and in their hybrids confirm Dunn's suggestion that the two conditions are associated, the amyloidosis preceding the nephritis. Both genetic and nongenetic factors are demonstrated to be causative agents. Segregation of genetic factors was evident in the F_2 and strain A back-cross generations. The occurrence of some nephritis, although of low incidence, in the genetically homogeneous groups of strain L and the F_1 hybrids resulting from crossing strain L with strain A was evidence of the influence of nongenetic factors.

Outstanding among the nongenetic factors was a chronic dermatitis caused by the mite Myobia musculi, and that resulted in secondary amyloidosis. The secondary amyloidosis in turn resulted in nephritis.

Segregation ratios in the F_2 and back-cross generations suggested that the primary amyloidosis and the resulting nephritis were caused by a single recessive gene. Final conclusions, however, must await the results of breeding tests of back-cross or F_2 segregants.

These results suggest the desirability of studies in search of such a genetic factor as the causative agent of primary amyloidosis in man.

10. Iverson, L., and Morrison, A. B.: Arch. Path. **45**:1, 1948.

VISCERAL LESIONS IN A CASE OF RHEUMATOID ARTHRITIS

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GRANULOMAS occurring in patients with rheumatoid arthritis have been examined by several investigators in order to demonstrate that the lesions are widely distributed throughout the body, and to compare these lesions with those of rheumatic fever. The subcutaneous nodules of both these diseases have been studied by Bennett, Zeller and Bauer,[1] who found many similarities, but also important differences, and concluded that "the nodules of rheumatoid arthritis and rheumatic fever differ as much from one another as do the granulomas of syphilis and tuberculosis." Granulomas have also been found in skeletal muscle[2] and along nerve trunks.[3] Evidence of granulomatous disease in the hearts of patients with rheumatoid arthritis has been of great interest in view of the similarity of this and rheumatic heart disease. Baggenstoss and Rosenberg[4a] frequently found lesions identical with the scars of rheumatic heart disease in cases of rheumatoid arthritis, without a history of rheumatic fever. In addition, the same authors[4b] also found in 2 cases a different cardiac manifestation of rheumatoid arthritis, namely, large granulomas with necrotic centers, closely resembling the subcutaneous nodules occurring in the same disease. Similar granulomas were also present in one of these 2 cases in the pericardium and in the nearby portion of the wall of the aorta. Other visceral lesions in rheumatoid arthritis have, to the best of my knowledge, not been reported.

In view of the importance of these observations for the understanding of rheumatoid arthritis as a generalized disease, and for the elucidation of the relationship of this disease and rheumatic fever, the following findings of granulomas in the pleura and the peritoneum are reported.

From the Departments of Pathology, Long Island College of Medicine and Kings County Hospital, Brooklyn.

1. Bennett, G. A.; Zeller, J. W., and Bauer, W.: Arch. Path. **30**:70, 1940.

2. Steiner, G.; Freund, H. A.; Leichtentritt, B., and Maun, M. D.: Am. J. Path. **22**:103, 1946.

3. Freund, H. A.; Steiner, G.; Leichtentritt, B., and Price, A. E.: Am. J. Path. **18**:865, 1942.

4. Baggenstoss, A. H., and Rosenberg, E. F.: (*a*) Arch. Path. **35**:503, 1943; (*b*) **37**:54, 1944.

REPORT OF CASE

A 57 year old white man was admitted to Kings County Hospital with a six year history of rheumatoid arthritis. The disease began with a painful, hot swelling of the right shoulder. Subsequently, other joints were affected in a similar manner. The patient lost 18 Kg. (40 pounds) during these six years and was completely incapacitated during the sixth year. Ten days before admission the right shoulder again became swollen and tender. On admission the temperature was 98.6 F., the pulse rate was 84, and the blood pressure was 160 systolic and 98 diastolic. The right shoulder, elbow and knee were swollen and hot, but not red. The shoulder was tender. All other joints which could be examined, with the exception of the left shoulder, were swollen but not hot. The fingers were deformed, and the interossei muscles were atrophied. During the patient's stay in the hospital a decubitus ulcer developed over the sacrum. His temperature rose to 104.6 F., and he died thirty-three days after admission.

Laboratory observations, made shortly after admission, included: hemogoblin, 12 Gm. per hundred cubic centimeters; erythrocytes, 3,900,000, with white blood cells, 10,600, per cubic millimeter; Wassermann test, negative; blood urea, 46 mg., creatine, 1.7 mg., and uric acid, 4.2 mg., per hundred cubic centimeters. The urine was cloudy; tests for albumin and glucose showed none. Twenty-three days after admission the white blood cells numbered 20,000 per cubic millimeter, 85 per cent of which were polymorphonuclear leukocytes, 13 per cent lymphocytes and 2 per cent monocytes. Roentgenograms showed, in addition to the typical lesions of the joints of the extremities, marked hypertrophic arthritis with subluxation of the third and following cervical vertebrae, and destruction of carpal bones.

Autopsy.—The anatomic diagnosis was as follows: rheumatoid arthritis of many joints; granulomas of rheumatoid arthritis in the right atrium, the tricuspid valve, the pleura and the capsule of the spleen; partial fibrous obliteration of pericardial and pleural cavities; generalized arteriosclerosis; old obliteration of the left circumflex coronary artery; scarring following myocardial infarction of the left ventricle; arteriosclerotic aneurysm of the abdominal aorta; arteriosclerotic scarring of the kidneys; small pulmonary embolus; pulmonary emphysema and atelectasis; cortical adenomas of the kidneys; concretions in the bladder; diverticulum of the bladder; cystitis; fibroadenomas of the prostrate; adenomas of the thyroid gland; decubitus ulcer over the sacrum. In the present discussion, only the findings of interest will be described.

Heart: The pericardial sac was partly obliterated by fibrous adhesions. The remaining cavity contained pale, turbid fluid. The free pericardial surfaces showed irregular hard thickenings and were covered with flakes of soft fibrin. The heart weighed 320 Gm. The chambers were opened in the usual manner. The right atrium showed on its inner surface in the area of the musculi pectinati a large number of yellowish white firm thickenings, measuring approximately 2 mm. in diameter. One leaflet of the tricuspid valve showed a pale, firm thickening, which measured 1 cm. in diameter and 5 mm. in thickness. The remainder of the tricuspid valve, as well as the pulmonary and mitral valves, were not remarkable. The walls of the right ventricle measured 3 mm. in thickness and showed no significant changes. The leaflets of the aortic valve showed numerous arteriosclerotic plaques. The circumferences of the tricuspid, pulmonary, mitral and aortic valves measured 14, 6, 11 and 7.5 cm., respectively. In the wall of the left ventricle, near the apex there was an area in which the myocardium was replaced by fibrous tissue. This portion of the wall was markedly thinner than the rest and measured 6 mm. in thickness, whereas the remainder of the wall meas-

ured 14 mm. All coronary arteries showed marked arteriosclerosis, and their walls were rigid. The lumens were wide and gaping; only the left circumflex artery was narrow and makedly calcified. It was completely obliterated about 1 cm. from its origin from the main artery.

Aorta: The entire aorta showed arteriosclerosis, which was especially severe in the descending portion. There were numerous large, firm plaques, some of them calcified or ulcerated. Below the level of the origin of the inferior mesenteric artery there was a spindle-shaped dilatation in which the diameter was twice that of the rest of the vessel. In this area there were numerous ulcerated plaques partly covered by thrombotic material which formed a layer 0.5 cm. in thickness.

Lungs: The left pleural cavity was obliterated by fibrous adhesions with the exception of a small pocket which contained fibrin and turbid fluid. The upper portion of the right pleural cavity was similarly obliterated, whereas the lower portion contained a yellowish, turbid fluid and large amounts of fibrin. The surface of the lung in this area showed irregular hard thickenings and was covered with masses of soft fibrin. The left lung weighed 470 Gm.; the right lung, 430 Gm. The anterior portions of both lungs were pale and emphysematous, whereas the posterior portions were red and firm. A large portion of the lower lobe of the right lung was firmly consolidated and reduced in size. Its cut surface was pale red. Several small calcified nodules were scattered irregularly throughout both lungs. The mucosa of all bronchi was red and swollen. A small embolus was found in an artery of the lower lobe of the left lung. The hilar lymph nodes were anthracotic.

Spleen: The spleen adhered to neighboring organs at several points. The greater part of its surface was covered with white fibrous tissue. The weight of the organ was 330 Gm.; the consistency was firm. On section the tissue was dark purple and showed distinct trabeculae and follicles.

Joints: Both sternoclavicular joints had enlarged cavities, which contained soft, pale yellow, fatlike material. The articular surfaces were rough. Of the larger joints, only the right shoulder was examined. The capsule of the joint was irregularly thickened. The articular surfaces showed many defects in their cartilaginous linings. A large cavity next to the joint was filled with a brown, viscid, somewhat turbid fluid. Several cervical vertebrae were compressed in a craniocaudal direction.

Microscopic Examination.—Tissues were fixed in Zenker's fluid and embedded in paraffin. Sections were stained with hematoxylin and eosin and, whenever desired, with any of the following methods: Masson's trichrome stain, Gömöri's silver impregnation of reticulum, Gram stain, and stain for acid-fast bacteria. Stains for bacteria revealed no bacteria and will therefore not be described.

The heart showed in the right atrium and in the tricuspid valve large granulomas, which were seen as nodules on gross examination. The muscular wall of the right atrium was completely replaced in some areas by this granulation tissue (fig. 1). A tpyical unit of the abnormal tissues (fig. 5) consisted of a granuloma with an area of necrosis in the center. The necrotic tissue stained red with hematoxylin and eosin and showed no cell bodies or nuclei. Only fine blue-staining particles were present, which were probably remnants of fragmented nuclei. In some of the necrotic areas these bluish-stained particles were limited to the border. The necrotic tissue was surrounded by the granulation tissue, in which two zones were distinguished, which were identical with those described by Baggenstoss and Rosenberg.[4b] The inner zone consisted of large oblong cells in a radial arrange-

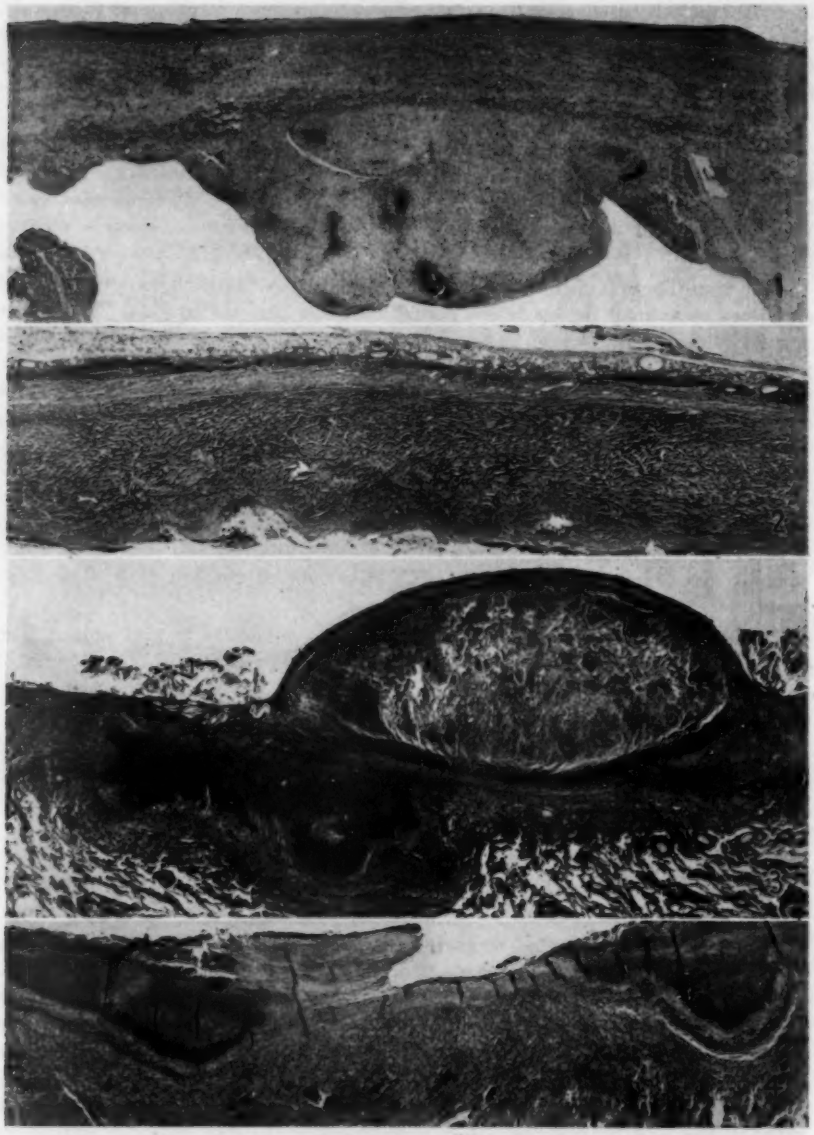

Fig. 1.—Right atrium showing a mass of granulation tissue protruding into the lumen. Note the several areas of necrosis in the granuloma. Hematoxylin and eosin stain; × 18.

Fig. 2.—Parietal pericardium with a layer of dense fibrous tissue. The lower surface in the figure faces the pericardial cavity. Masson stain; × 18.

Fig. 3.—Lung with several large granulomas in the pleura. The node protruding on the surface is not surrounded by granulation tissue. The other node, located deeper and to the left of it, contains dark-staining, more recent areas of necrosis surrounded by granulation tissue. Hematoxylin and eosin strain; × 18.

Fig. 4.—Surface of the spleen with a layer of granulation tissue. The necrotic portion of the latter is on the surface; the granulation tissue, between it and the pulp. Hematoxylin and eosin stain; × 18.

ment. These cells had large, pale-stained nuclei, which might be rod shaped or somewhat lobulated. Interspersed between the cells were polymorphonuclear leukocytes and lymphocytes. Some of the large cells had apparently fused with one another and given rise to giant cells. The arrangement of the nuclei of some of these giant cells was irregular; in others the nuclei were arranged in the form of a sphere as is characteristic of the Langhans giant cell. The outer layer of granulation tissue had an uncharacteristic appearance and consisted of fibroblasts with large nuclei resembling those of the cells in the inner zone, lymphocytes and small numbers of polymorphonuclear leukocytes. This granulation tissue blended without a definite border into that of nearby nodules or into the surrounding connective tissue. The development of the granulomas appeared to be in different stages at various parts of the circumference. In some areas, the border of the necrotic zone showed many nuclear fragments, and the adjacent palisading cells were well developed. In other areas, these cells could hardly be distinguished, and the necrotic tissue was almost in contact with the outer layer of granulation tissue. The large node in the tricuspid valve showed particularly little growth activity. In some portions of its circumference the granulation tissue was almost absent, and in others it was reduced to a row of large foam cells. Some of these foam cells were multinucleated, which suggested that they had arisen from the large palisading cells of the granulation tissue. The nuclei of the small foam cells also resembled those of the cells in the granulation tissue. There was but minimal lymphocytic and plasma cell infiltration. Mallory's stain for collagenous fibers and Gömöri's silver impregnation for lattice fibers revealed large numbers of both types of fibers in all layers of the granulomas. The arrangement of these fibers was more clearly seen in granulomas of the spleen and will be described there.

Several sections of the visceral and the parietal pericardium showed diffuse inflammation. In some areas there was a granulation tissue of uncharacteristic appearance which contained foam cells resembling those seen in the tricuspid valve. In other areas there was a thick layer of dense fibrous tissue which showed on the surface alternating elevations and depressions (fig. 2). An arrangement of the coarse fiber bundles in whorls was apparent at many points, resembling that in the center of the large granulomas. In one of these areas in which the pericardial lumen was obliterated except for a few small clefts lined by cuboidal epithelium, the fibrous tissue was covered by a thin layer of granulation tissue. The cells closely resembled those in the granulomatous nodules, and a few large multinucleated cells were also present (fig. 6). The arrangement of the connective tissue fibers and the presence of this granulation tissue suggested that in these portions of the pericardium a process had occurred which was similar to that in the nodular granulomas except that it was spread out along a serous membrane rather than concentrated in the form of nodules.

The aorta showed severe arteriosclerotic changes, which are not to be described here. Only one observation must be mentioned. The adventitia showed an area of thickening and fibrosis which somewhat resembled a granuloma. The center of this area contained few cells, and in some portions it was entirely acellular. Surrounding this center was an uncharacteristic granulation tissue, which consisted mostly of small fibroblasts and a few round cells. This nodule was distinctly different from the characteristic granuloma of rheumatoid arthritis, and their relation could not be ascertained. Several other sections of the aorta showed no similar nodules.

The lungs were the seat of numerous areas of acute and chronic inflammation which is of no concern here. The pleura was thickened by the presence of granulation tissue and fibrosis, and in many areas these changes were of the usual

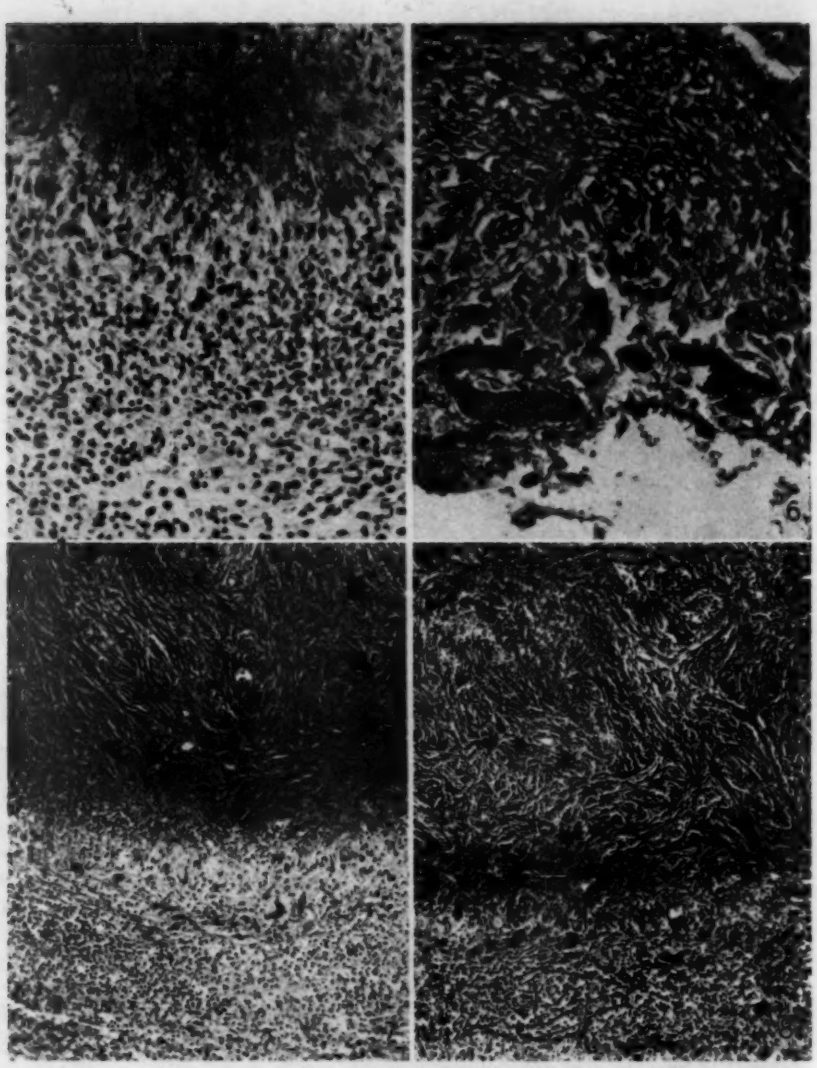

Fig. 5.—Right atrium, showing with higher magnification one of the areas of necrosis seen in figure 1. The nearby granulation tissue is seen in the lower portion of the figure. Hematoxylin and eosin stain; × 250.

Fig. 6.—Pericardium showing the border of the fibrous layer (see figure 2) lined by granulation tissue resembling that of the nodular granulomas. Hematoxylin and eosin stain; × 280.

Fig. 7.—Portion of the layer of granulation tissue on the surface of the spleen (see figure 4). The upper part of the figure shows the area of necrosis; the lower part, the granulation tissue proper. Hematoxylin and eosin stain; × 140. (The dark spots in the upper part are artefacts.)

Fig. 8.—Section of the same block as the section in figure 7, with Masson stain; × 140.

nonspecific appearance. In other parts there were large granulomatous nodes which closely resembled those found in the right atrium (fig. 3). They also showed single or confluent areas of necrosis surrounded by a granulation tissue with fairly numerous giant cells. It was common to find granulation tissue lining only that side of the necrotic areas which faced the lung tissue, whereas the other side was adjacent to fibrous tissue. At one point the layers of the granulation tissue were arranged parallel to the surface of the organ rather than in the form of a node. The necrotic portion was nearest the surface, and the granulation tissue was found between it and the lung tissue. This may well have represented an earlier stage of the fibrosis described in the pericardium. The necrotic tissue in a large group of confluent nodes contained a large amount of anthracotic pigment. Another node was distinguished by the complete absence of granulation tissue. The necrotic area was surrounded by fibrous tissue everywhere (fig. 3). This resembled somewhat the node found in the tricuspid valve.

The spleen showed extensive thickening of its capsule due to the presence of granulation tissue both in the form of nodules and of layers parallel to the surface (fig. 4). In both instances there was the same sequence of necrotic tissue, palisading large fibroblasts and uncharacteristic granulation tissue (fig. 7). The arrangement of the connective tissue fibers in the necrotic areas was similar to that seen in the heart and the pleura, but its features were best recognized in sections of the spleen. As has been mentioned, stains for collagenous and lattice fibers revealed large numbers of both (fig. 8). They formed bundles, which were arranged irregularly or in whorls. The interior of the spleen showed nothing of interest.

The head of the humerus showed an irregular articular surface, which was covered by cartilage only in some scattered areas. At other points the marrow spaces were separated from the joint cavity only by thin trabeculae of bone and a narrow layer of connective tissue. The synovial membrane was thickened by the presence of a nonspecific granulation tissue which contained large numbers of foam cells. No typical granulomas were present, but in a few areas the foam cells formed small nodules which also contained small round cells and moderate numbers of fibroblasts. In the center of one of these nodules there were a number of foam cells which were large and multinucleated. Within the bone of the humerus there were several nodules consisting of large fibroblasts and foam cells, and between them spindle-shaped cavities which had the shape of cholesterol crystals. In several of these nodes a central area was occupied entirely by debris with spindle-shaped cavities.

COMMENT

There is no direct proof that in the present case the visceral lesions are those of rheumatoid arthritis. However, this relationship is suggested by the fact that the patient had rheumatoid arthritis, as well as by the close resemblance of the granulomas in the viscera to those commonly seen in the subcutaneous tissue in rheumatoid arthritis. Furthermore, the granulomas observed in the heart conformed with those previously found in 2 cases of this disease by Baggenstoss and Rosenberg. Staining methods revealed no bacteria in tissue sections. For these reasons it will be assumed in the following discussion that the lesions under consideration are part of the generalized disease associated with rheumatoid arthritis.

It has been the purpose of this report to demonstrate the hitherto unknown fact that granulomas of rheumatoid arthritis may be located in the pleura and the peritoneum. Since similar granulomas had previously been found in the pericardium,[4b] and since there were also indications that a similar process may have gone on in the pericardium in the present case, the observation that such granulomas occur in all serous membranes is now on record. There were shown in the present case, also, granulomas in the heart which conformed with those described by Baggenstoss and Rosenberg[4b] except that they were located in the right side of the heart.

It is noteworthy that the granulomas in question, which largely conformed in their structure to those described in the subcutaneous tissue and in the heart, are often found in approximately the same condition. This suggests, as Bennet, Zeller and Bauer[1] have stated, that the process of development of these nodules is a slow one. Only one of the nodules in the tricuspid valve and one in the pleura of the present case differed from the others by showing no border of palisading cells and granulation tissue. In the typical granulomas, necrosis in a large central area affects a preexisting granulation tissue rather than a previously normal tissue, as can easily be demonstrated by stains for connective tissue. The fibers which are demonstrated by these methods are arranged in whorls and show no indication of the structure of the previous normal tissue in that area. It is peculiar that these staining qualities persist in the necrotic centers of all nodules even though some of these are presumably of long standing.

There are indications that a type of growth similar to that of the nodules may also occur in a continuous layer spread out over large areas of serous surfaces. In that event the various layers of the granuloma are arranged roughly parallel to the surface of the organ rather than concentrically. It is probable that in a late stage of development these formations give rise to a thick layer of firm fibrous tissue in which the original whorl-like arrangement of the fibers can still be seen.

A few previous reports may be mentioned which possibly deal with changes similar to those in the present case. Bennett, Zeller and Bauer[1] referred in passing to a case in which lesions resembling the subcutaneous nodules of rheumatic fever (as opposed to rheumatoid arthritis) were found in the pericardium and in the pleura of a patient with rheumatoid arthritis. Fingerman and Andrus[5] studied the visceral lesions in 61 cases of rheumatoid arthritis and found hyaline perisplenitis in 8 cases and marked fibrous pleural adhesions in 23. The distribution of these lesions corresponds well with that of the granulomas in the present case, and this is significant since dense fibrosis of the pericardium in the present

5. Fingerman, D. L., and Andrus, F. C.: Ann. Rheumat. Dis. **3**:168, 1943.

case showed indications of being derived from the characteristic granulation tissue of rheumatoid arthritis.

SUMMARY

Autopsy observations in a case of rheumatoid arthritis of six years' duration are described with particular reference to visceral lesions. Granulomas identical with those known to occur in the subcutaneous tissue and in the left side of the heart were found in the right side of the heart, the pleura, and the capsule of the spleen. This indicates, in conjunction with previous reports, that all serous membranes are subject to lesions of rheumatoid arthritis, as well as both sides of the heart and other previously known locations.

Case Reports

EWING'S ENDOTHELIAL MYELOMA OF ADOLESCENTS

Report of Two Fatal Cases

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ABOUT a quarter of a century ago Ewing[1] described a tumor of bone which he called "diffuse endothelioma of bone." The condition is found mainly in children, with the first evidence of the disease occurring usually as fever, pain, tenderness and leukocytosis. It is often confused with osteomyelitis. The primary site is frequently in the diaphysis of one of the long bones. There are marked osteolysis spreading along the shaft of the bone, expansion and perforation of the cortex, and a process extending along the periosteum into the soft tissues. The neoplasm probably spreads by the blood stream but may involve regional lymph nodes. Other bones become involved either through metastasis or, as some believe, through multicentric development of the disease.[2] Eventually metastases are found, usually in the lungs and occasionally in many organs. The tumor is temporarily radiosensitive, but radiation offers no cure for Ewing's tumor. The present view favors considering the disease as cancer of the reticuloendothelial system, a view which is not far removed from the original concept held by Ewing. Specimens of the tumor vary somewhat in cytologic detail but present a fairly distinct clinical entity.

REPORT OF CASES

CASE 1 (reported with the permission of J. D. Brown, M. D., and R. R. Morrall, M. D.).—R. J., a 13 year old white boy, first noticed pain in his right thigh in the early days of August 1945. The pain was severe at night and kept him awake. August 9 he was playing baseball when, without any direct trauma, his leg gave way and he fell. On being admitted to the Youngstown Hospital he was found roentgenologically to have extensive medullary and focal cortical absorption of the right femur, with a pathologic fracture near the junction of the middle and upper thirds of the bone. There were also periosteal elevation and proliferation along the shaft of the bone for several inches proximal to the fracture. The

1. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

2. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: Debatable Tumours in Human and Animal Pathology, London, Oliver & Boyd, Ltd., 1940.

roentgenologic findings were interpreted as characteristic of Ewing's tumor. No other bones were found involved at that time. A few days later a biopsy confirmed the diagnosis of Ewing's endothelial myeloma. During the early days in the hospital there was occasional mild fever, the temperature rising to a maximum of 101 F. The pulse rate corresponded, with mild elevation.

The erythrocyte count was 3,950,000; the hemoglobin content was 11.5 Gm. The leukocyte count was 5,800, with a differential count showing 70 per cent polymorphonuclears and a slight shift to the left. The serum phosphorus was 4.9 mg., and the blood calcium 10.397 mg., per hundred cubic centimeters. The Kahn and Kline tests revealed no syphilis. The urine had a specific gravity of 1.027; it contained no albumin, no sugar and no Bence Jones Protein.

Roentgen therapy was given to the maximum tolerance—a total of 8,496 roentgens. By the third month there was partial healing of the fractured bone. By the fifth month pain had developed in the frontal region of the head and in the left mandibular region. He was transferred to the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York, for several weeks. There he received seventeen injections of Coley's toxins (an unfiltered mixture of erysipelas and Bacillus prodigiosus cultures) and additional roentgen therapy. He was readmitted to the Youngstown Hospital in the eighth month after symptoms had begun. Pain was generalized by that time in both extremities and in his head. The blood pressure became elevated to 160 systolic and 100 diastolic. There was little or no fever. Morphine had to be given daily for severe pain. By the eleventh month he was completely blind in the left eye, and vision was reduced in the right eye. He became emaciated and weak and died about fourteen months after the appearance of the initial symptoms.

Autopsy.—The body was that of an emaciated 14 year old boy. There was a tumor involvement of the upper part of the right femur, the skull, the right clavicle, both tibias, the left fibula and the left second rib. Also involved were soft tissues as follows: both lungs, the small intestine, the pancreas, the mesenteric lymph nodes, both kidneys, one adrenal gland, the dura mater, the leptomeninges over the cerebrum and, slightly, the outer part of the cerebrum. Both lungs showed bronchopneumonia. Histologically, the tumor was fairly uniform in the various sites where it was found. The cells were found in solid sheets or masses; they were fairly large cells, often rounded, with large hyperchromatic nuclei, which were frequently vesicular and occasionally showed a nucleolus. The nuclear border was usually well defined, and the cytoplasm of the cell was scanty. In many nuclei the chromatin was clumped. There was little intercellular substance in most masses of the tumor. Some of the lobules of the tumor were separated by a small amount of connective tissue. Silver stains showed no reticulum fibrils in close relationship to the tumor cells. In some masses of the tumor there was striking rosette arrangement of the tumor cells, best seen in the masses found in the lungs. The rosette was composed of a central pale fibrillar mass surrounded by roughly radiating cells. No blood vessels could be detected in these central zones in most places. Vascularization was abundant in most of the neoplastic masses, with many areas showing collections of blood or blood sinusoids lined by tumor cells. Occasional veins showed tumor thrombi. No tumor was evident in the spleen, but in the spleen there was extensive evidence of destruction of blood, with many histiocytes loaded with iron-bearing blood pigment. The tumor which remained in the right femur was altered considerably by necrosis, apparently caused by the heavy roentgen irradiation.

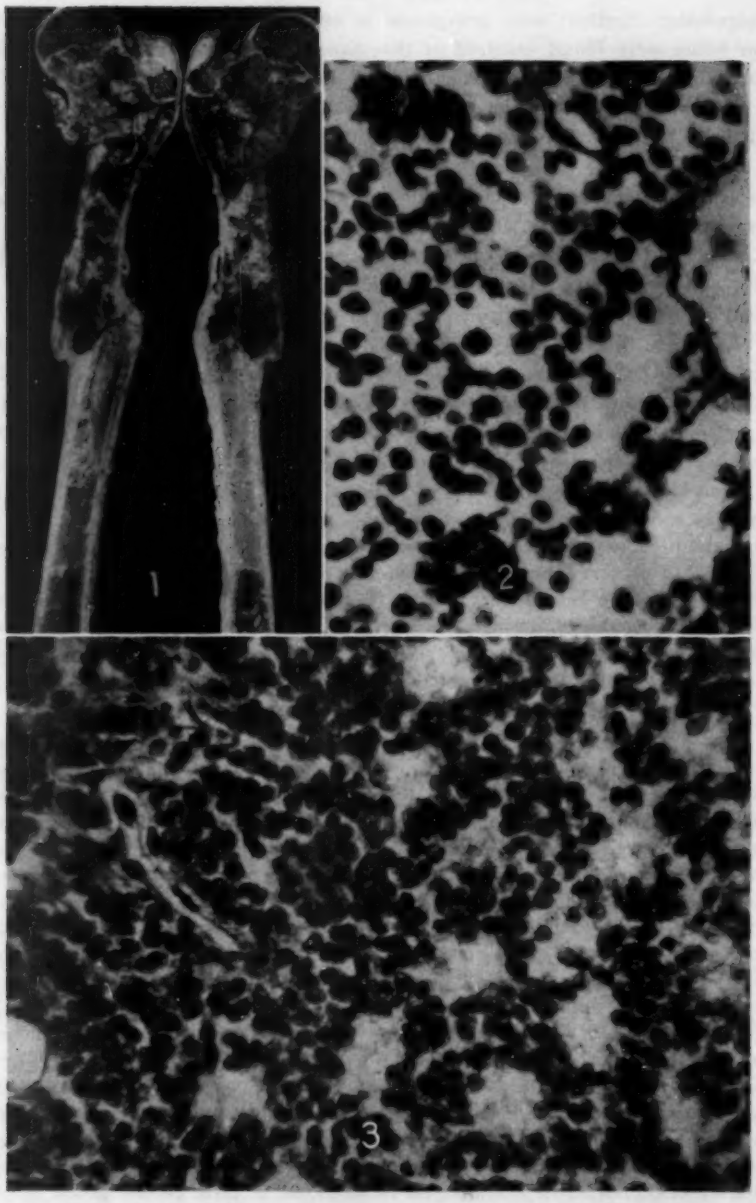

Fig. 1 (case 1).—Femur as seen at death. It shows extensive neoplastic involvement above and below the fracture, which is partially healed.

Fig. 2 (case 1).—Metastatic tumor of the lung showing abundant rosette formation and some condensation of tumor cells about blood vessels.

Fig. 3 (case 2).—Pulmonary tumor nodule stained with silver. There is no reticulum in most of it and none intimately related to the tumor cells. Cytoplasm is scanty.

CASE 2 (reported with the permission of R. R. Morrall, M.D.).—A 16 year old white girl first felt discomfort in her left shoulder while playing baseball about May 1, 1946. She did not remember that her shoulder was actually injured, but pain began at that time. She was treated by a chiropractor for about two months. The soreness in her left shoulder was poorly localized. When she was admitted to the Youngstown Hospital, July 29, there was a swelling which seemed to elevate the left scapula. Roentgenologic examination revealed a zone of irregular destruction of bone in the body of the left scapula with some elevation of the regional periosteum. A biopsy showed Ewing's tumor with invasion of the surrounding muscles.

The erythrocyte count was 4,890,000; the hemoglobin content was 14.5 Gm. The leukocyte count was 4,450, with a normal differential count. The serum phosphate was 4 mg. per hundred cubic centimeters, and the serum phosphatase, 4.9 units—both within normal limits. The Kahn and Kline tests of the blood revealed no syphilis. The urine contained no albumin, sugar or cells, and no Bence Jones protein was evident.

On examination no other bone could be detected in which there was any abnormality. After a biopsy, heavy roentgen radiation was applied locally over the left scapular region. The patient seemed to improve clinically during the first few weeks. However, about three months after the initial symptoms had been noted, she began to experience discomfort around her right knee. Soon discomfort developed in the lower lumbar region of the back. By the fourth month she had a low grade fever, but the blood count and the urine remained normal. A roentgenogram revealed focal bony destruction in the third lumbar vertebra. She lost weight rapidly, and a soft tissue mass began to develop over the left midparietal area of the skull. Soon roentgenograms showed osseous destruction in this region and also in the distal end of the right femur. Generalized pain became marked. Both eyes became swollen, and blindness developed in the left eye. She died about six months after she had noted the first symptoms.

Autopsy.—The body was that of a well developed but emaciated 16 year old white girl, with a pale skin. There were petechiae over the thorax and the abdomen. A tumor involved the left scapula, the skull on both sides, several ribs, thoracic and lumbar vertebrae and the right femur. There were metastases of the tumor in both the lungs and the pleurae, in the pancreas, in the pituitary gland and in the dura mater and the leptomeninges, with slight invasion of the upper part of the cerebrum. Histologically, the tumor was found in solid sheets, rounded, compact nodules, or strands. The cells were moderately large and rounded or pleomorphic; they had scanty cytoplasm and round or oval nuclei, which were often vesicular. The chromatin was irregular in distribution, and the nucleoli were inconspicuous. Tumor thrombi were scattered in the lungs, and solid masses of neoplasm filled many of the alveoli. There was little tendency to form rosettes in this tumor. In most places, intercellular substance was lacking, and reticular stains revealed no intimate relationship between the interlobular connective tissue reticulum and the tumor cells themselves. Scattered hyperchromatic nuclei were present, and a few atypical mitoses were seen in some nodules. The masses of tumor cells showed marked vascularization, with many blood sinuses lined by tumor cells.

COMMENT

These 2 cases of Ewing's tumor are fairly typical. Both patients were adolescents. Both related their first symptoms to playing baseball, but

neither showed clear evidence of direct trauma. In the boy the original pain, swelling and bony lesion were in the femur, while in the girl the neoplasm seemed to originate in the left scapula. In both cases there was an interval of several weeks or months before lesions were evident in other bones or tissues. Both patients had been in apparently good health prior to the onset of the disease.

Ewing believed that the tumor originated from the endothelium of the medullary capillaries of the bones. Present writers definitely relate it to the reticuloendothelial system, and therefore their observations are not far removed from Ewing's original concept. Hadfield[3] classed Ewing's tumor under reticulosarcoma and considered it along with some other tumors as undifferentiated mesenchymal tissue found in syncytial arrangement or as tumors derived from the primitive mesenchyme which show varying degrees of differentiation. Some of the tumors show reticulin fibrils; some seem to show immature lymphocytes or lymphoblastic tissue and some show sheets of cells with little evidence of differentiation. Ewing's tumor is osteolytic but does not produce bone in itself. It does penetrate the cortex and tends to expand it. It may cause considerable proliferation and slight new bone formation in the subperiosteal region of the involved primary site. In some instances it is composed of larger cells than the ones illustrated in our cases. Hadfield described the characteristic cells as having copious, faintly staining cytoplasm and a vesicular nucleus such as is seen in the reticulum cells. Usually the fairly uniform cells are closely packed, with little intercellular substance. Apparently Oberling[4] was the first to relate the tumor to the reticuloendothelial system. He defined four different types showing various degrees of differentiation.

Willis[5] suggested that at least in some of the clinical cases Ewing's tumor may be secondary to an inconspicuous primary focus of neoplastic growth in one of the internal organs. It may originate in the adrenal gland, in nerve tissues or in the lungs. It is true that the tendency to form rosettes in many of the cases suggests a neurogenic type of tumor. In our cases there was found no evidence to substantiate the belief that the origin of the tumor was at any site other than that described. The fact that swelling and pain occurred at the site of the original lesion seems to indicate that this was the true site of origin. One of our patients did have a tumor metastasis in the cortex of one of the adrenal glands, but

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3. Hadfield, G., and Garrod, L. P.: Recent Advances in Pathology, Philadelphia, The Blakiston Company, 1947.

4. Oberling, C.: Bull. Assoc. franç. p. l'étude du cancer 17:259, 1928.

5. Willis, R. A.: The Spread of Tumours in the Human Body, London, J. & A. Churchill, Ltd., 1934.

no evidence could be found to indicate that the tumor was in either the medulla or any other part of the nervous system.

Phemister[6] attempted to separate Ewing's sarcoma from reticulum cell sarcoma, angiosarcoma and lymphosarcoma occurring in the bones. The first two and the last, however, all are seen most frequently in children and seem poorly defined from his description and illustrations. It seems reasonable to class them together as varying only in the degree to which they have differentiated from the multipotential mesenchyme from which they apparently originate.

SUMMARY

Two fairly typical cases of Ewing's tumor are presented, with the clinical, histologic and radiologic characteristics. The tumor is accepted as a cancer of that part of the reticuloendothelial system which is located in the medullary regions of bones, especially in those of the diaphyses of long bones.

6. Phemister, D. B.: J.A.M.A. **136**:550, 1948.

DYSCHONDROPLASIA WITH HEMANGIOMATOSIS (MAFFUCCI'S SYNDROME) AND TERATOID TUMOR OF THE OVARY

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THE COMBINATION of dyschondroplasia and hemangiomatosis is exceedingly rare. Carleton and associates[1] were able to collect 18 cases from the European literature, and to this group they added 2 cases of their own. Krause[2] recorded the first case to be described in the American literature, in 1944. Carleton and associates[1] suggested that the name of Kast[3] be associated with the syndrome. However, in 1942, after a further study of the literature, they discovered that the first recognizable case of dyschondroplasia associated with hemangiomatosis was described in 1881 by Maffucci, and his name was then given to the syndrome.

Dyschondroplasia, or Ollier's disease, is a condition affecting the growing ends of the bones. Normal ossification of cartilage does not take place, and as the bone increases in length, the areas of cartilage which fail to ossify persist in the metaphysis. There is dwarfing of the limbs, with irregular bending, and there are multiple nodular tumors. The condition is commonly unilateral or markedly asymmetric. Maffucci's syndrome differs from Ollier's disease in that there is present the additional element of hemangiomatosis.

Dyschondroplasia can be regarded as a nonhereditary mesodermal dysplasia which becomes clincially evident before puberty. The reported cases concerned 15 males and 6 females. The patients' ages at the dates of recording ranged from 8 to 58 years—with an average of 33 years. According to the report of Carleton and associates,[1] the usual history is as follows: The child is apparently normal at birth, but sometime in the years before puberty, from the first to the twelfth year, a hard nodule, 1 to 2 cm. in diameter, appears on a finger or a toe to be followed soon afterward by other nodules on the feet and the hands and on the legs and arms. The vertbrae, the ribs, the scapulas and the pelvis may be the

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1. Carleton, A.; Elkington, J. St. C.; Greenfield, J. G., and Robb-Smith, A. H. T.: Quart. J. Med. **11**:203, 1942.

2. Krause, G. R.: Am. J. Roentgenol. **52**:620, 1944.

3. Kast and von Recklinghausen: Virchows Arch. f. path. Anat. **118**:1, 1889.

sites of tumors, but the skull, the insteps and the wrists are rarely involved. These hard nodules are identified as enchondroma. At the time at which they appear, soft, bluish tumors (hemangioma) develop on the affected limbs. Large, dilated veins may be found associated with the soft tumors.

The distribution of the tumors (enchondroma and hemanigoma) may be extremely asymmetric, but it is rarely absolutely unilateral. Skeletal development may be retarded on one side; one or more long bones may have short shafts with irregularly expanded ends and cartilaginous tumors at the epiphysial lines. Trivial injuries may cause fractures of the long bones, with delayed callus formation or nonunion.

The deformities may increase throughout the period of development, and in severe cases the hands and the feet may become transformed into such huge enchondromatous masses as to be almost unrecognizable. The patient presents an extremely grotesque appearance, and the affected limb or limbs may be so large that all function is lost, and amputation becomes imperative for comfort.

In the early twenties the condition becomes stationary, and if there has been only a moderate degree of deformity, which may necessitate only loss of a finger or a toe, the affected person can be considered fortunate. In several instances, however, injuries occurring later in life have caused new nodules to appear. There is no pain associated with either the hard or the soft tumors.

Amputations were carried out in almost one half of the reported cases. Chondrosarcoma was noted in 4 instances. Other reported changes included abnormal sweating, vitiligo and cerebral glioma.

REPORT OF A CASE

S. S. was a 19 year old dwarfed white girl of Polish descent, not married. She presented herself in June 1943 with a gradually enlarging abdominal mass, the presence of which was associated with amenorrhea. In addition, there were long-standing irregular nodular enlargements of the distal portions of the extremities.

The parents and the siblings (five) had no deformities. There was no history of similar enlargements occurring in any of the near relatives. The patient's infancy was uncomplicated. At the age of 5, bowing of the legs was noted, and a diagnosis of "rickets" was made. The bowing, however, was peculiarly asymmetric, involving mainly the left side. At the age of 9, the proximal epiphysis of the left leg was subjected to epiphysial stimulation operation. The right leg had a corresponding epiphysial arrest operation. At the age of 11, the distal epiphysis of the left forearm was subjected to epiphysial stimulation. It is apparent, therefore, that the bone lesions were the first to occur. The first superficial nodule was noted on the dorsal surface of the distal phalanx of the right ring finger at the age of 14 years. It was followed rather quickly by other nodules, especially on the left hand and forearm and the left foot. The first nodule was bluish, compressible and soft, and others were of similar character, but the later prominent phalangeal enlargements were due to hard, fixed tissue.

Menstruation began at the age of 15, recurred at regular intervals and lasted four to five days. At the age of 19 years, or eight months before the patient entered the hospital, there was an abrupt cessation of menstruation. There was

gradual painless enlargement of the abdomen. This development had been known to the patient for a period of three to four months and was associated with the eight month period of amenorrhea.

The picture presented was that of a bowlegged white woman, 4 feet 7 inches (139.5 cm.) tall, with right dorsal scoliosis, compensatory lumbosacral scoliosis,

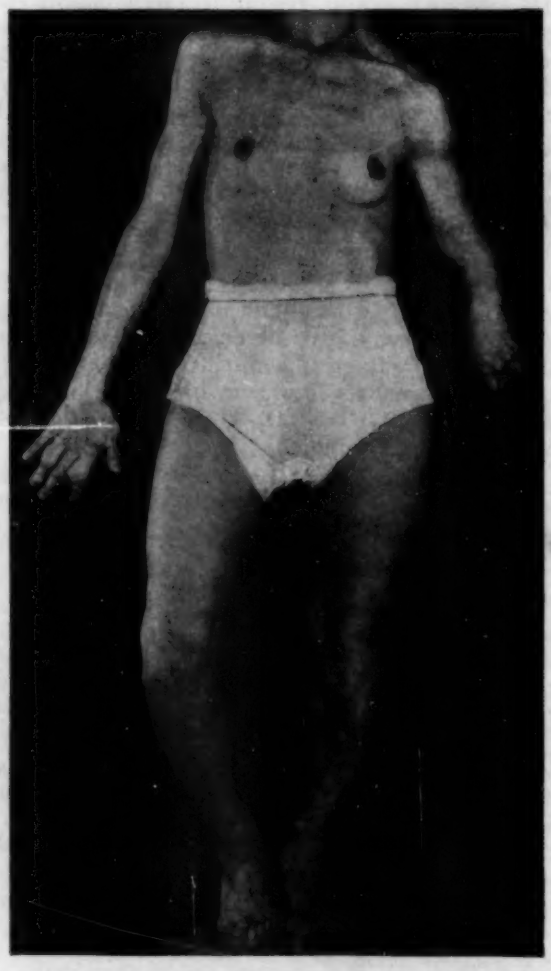

Fig. 1.—The photograph shows the deformity of the left shoulder and arm, the nodules of the digits, the genu varum and the attitude of scoliosis.

dropped left shoulder, short left arm and forearm, short left leg and multiple cutaneous and phalangeal nodules (fig. 1). The secondary sexual characteristics were well developed, although there was slight enlargement of the clitoris, as well as hypertrichosis of the legs. The head and the neck were normal.

Noteworthy physical findings were the orthopedic deformities and the abdominal mass.

The right arm was normally developed, and the finger tips reached the distal third of the femur. There were compressible bluish nodules on the dorsum of the wrist, the base of the thumb and the lateral aspect of the index finger at the distal end of the proximal phalanx, a fusiform swelling of the proximal phalanx and a cherry-like lesion of the nail bed of the ring finger. The nail of this finger was deformed. The veins of the hand and forearm were quite prominent, and two of the nodules at the wrist were situated along the course of a vein (fig. 2).

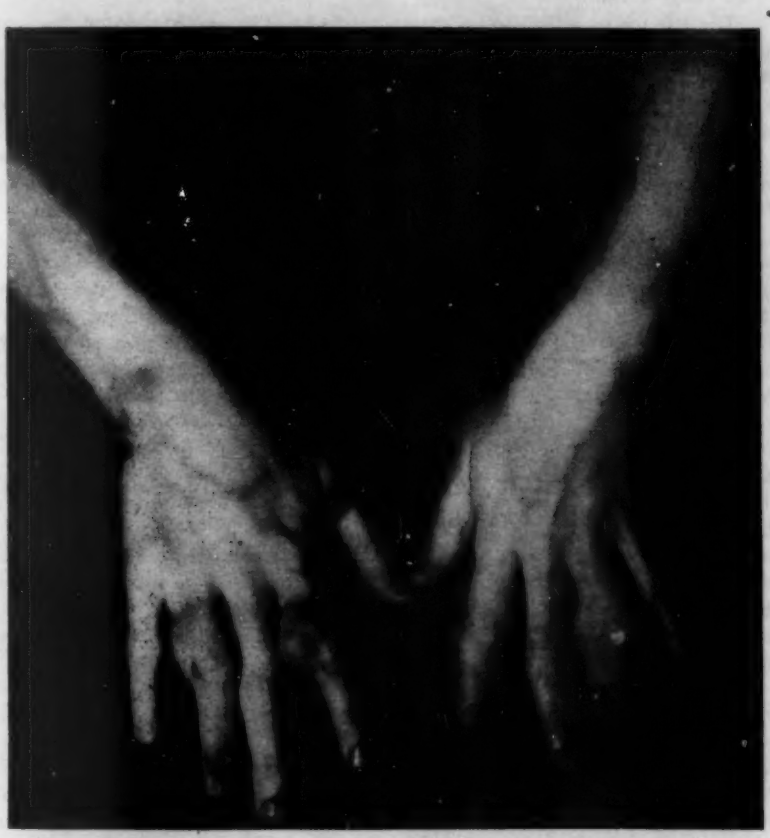

Fig. 2.—This infra-red photograph illustrates the vascular lesions of both hands and the prominent dilated venous system of the right hand and arm, along the course of which are two dark cutaneous nodules.

These nodules ranged from 1 to 2 cm. in diameter. The right leg presented marked genu varum, an old anterior tibial scar, with bluish red, soft nodules, 1.5 cm. in size, at the base of the second and third toes on the plantar aspect. There was dorsal crowding of the second toe, also flatfoot.

The left arm was quite short. Both the upper and the forearm were markedly shortened, so that the finger tips reached to the level of the femoral trochanter. The shoulder area presented a flattened deltoid region with the previously mentioned drop shoulder. There was a rather prominent valgus deformity of the elbow with marked lateral bowing of the forearm. The forearm was exceedingly short

and had a surgical scar at the distal ulnar epiphysis. The left hand was considerably smaller than the right and more prominently deformed. The largest of the multiple nodules was located at the proximal interphalangeal joint of the fourth finger. Other nodules were located at the distal end of the same finger and at the lateral margins of the other fingers, including the thumb. There were no palmar nodules in either hand. These nodules of the left hand were of mixed character. Some were bluish red and compressible, while others were quite hard and fixed to the bony substance of the finger.

The left femur was rather short and straight. The left trochanter was higher than the right and there was a tilt of the pelvis. The left leg revealed prominent lateral and anterior bowing. Multiple compressible soft bluish nodules were located along the anterior tibial line and prominently along the medial and lateral aspects of the ankle and heel. There was flatfoot of this extremity also.

Pelvic-rectal examination revealed a hard, smooth, nonfixed mass occupying the pelvis and extending up to a point 3 cm. above the umbilicus. It was the size of a five month pregnancy.

Roentgenograms of the right arm showed soft tissue nodules containing circumscribed radiopaque particles. There were no gross distinctive changes of the bony structures. The right foot showed radiolucent cystic areas in the great toe. There was a small radiolucent cystic area without disturbance of the cortex involving the subtrochanteric area of the femur. The left side presented multiple expansile cystic lesions of the clavicle, the scapula, the humerus, the radius, the ulna, the metacarpals and the phalanges. There was marked disturbance of the proximal end of the humerus and the proximal and distal portions of the radius (fig. 3*A*). The left wing of the ilium, the ischial rami and that part of the femur just below the trochanter exhibited similar radiolucent cystic areas (fig. 3*B*). The distal portion of the femur and the proximal portions of the tibia and the fibula were similarly involved. The phalanges of the left foot were the seats of punched-out cystic areas. There were a number of the calcified particles in the soft tissue nodules. Discrete radiolucent areas were noted in the ribs and in the right shoulder girdle. There was also the scoliosis mentioned. Roentgenograms of the pituitary area showed an irregular expanded outline of the sella turcica, suggesting a pituitary tumor.

There was moderate hypochromic normocytic anemia with considerable activity of the marrow. The blood sedimentation rate was 31 mm. in one hour and 61 mm. in two hours (Westergren). The serum proteins totaled 7 Gm., with albumin 5 Gm. and globulin 2 Gm., per hundred cubic centimeters. Acid phosphatase amounted to 4.6 and alkaline phosphatase to 13.8 King-Armstrong units. Blood calcium was 12.1 mg., phosphorus 3.2 mg., cholesterol 189.3 mg. and nonprotein nitrogen 27.3 mg. per hundred cubic centimenters. The Friedman test for pregnancy gave a negative result.

The left ring finger was amputated at the metacarpophalangeal joint to relieve the discomfort caused by the large tumors. Several large tumors (hemangioma) were removed from the left plantar surface. These tumors had recently grown so large that a shoe could not be used, and locomotion was difficult.

Histologic examination of the bony tumors of the amputated left fourth finger showed a rather young myxomatous cartilaginous tissue. This tissue occupied the central portions of the phalanges to the exclusion of the bony substance. There were minute irregular spicules of bone about the periphery. In some instances clusters of large cartilaginous cells were found in large, round spaces. The picture was that of a mixed myxomatous and hyaline cartilaginous substance. Articular surfaces were intact.

The bluish cutaneous nodules and the deeply situated colorless tumors were examined, specimens being taken from different parts of the body. The bluish nodules consisted of numerous vascular spaces with a variable degree of cellularity. In most instances the pattern was that of a cavernous hemangioma; in other

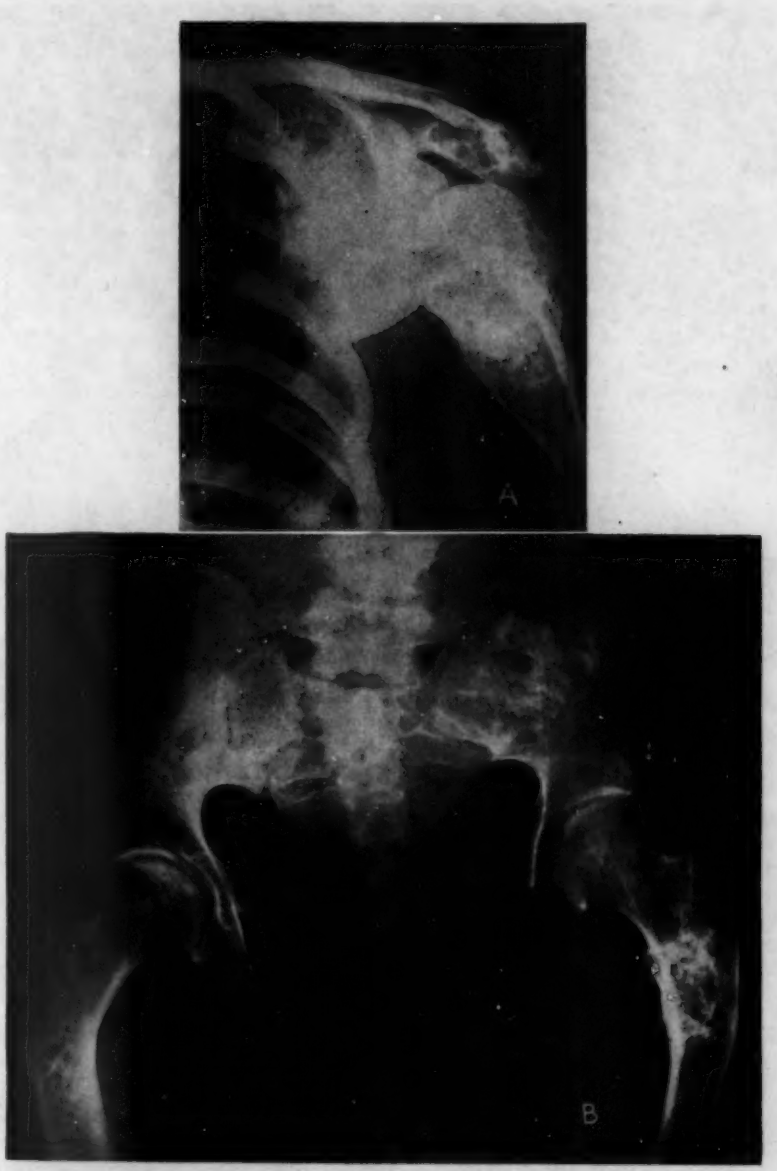

Fig. 3.—*A*, roentgenogram showing the radiolucent expansile cystic lesions of the proximal portion of the humerus, of the clavicle and of the scapula. *B*, roentgenogram showing involvement of the left wing of the ilium, of the ischiorami and of the subtrochanteric areas of the femurs.

nodules, however, there was a cellular growth with sparse, minute, poorly defined vascular spaces. In the cellular areas, occasional mitoses were evident in the round or somewhat elongated, finely granular nuclei.

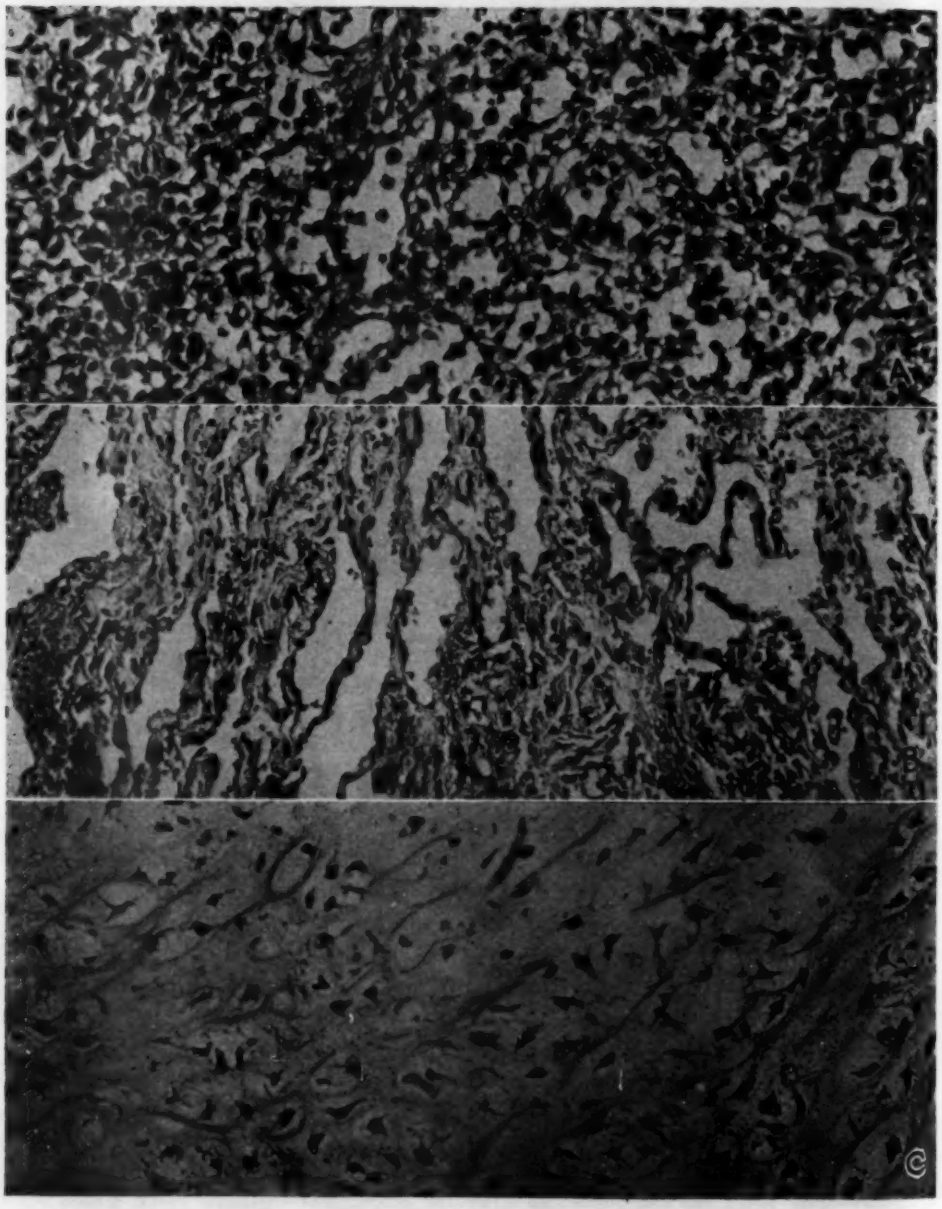

Fig. 4.—*A*, tumor. *B*, hemangioma of the skin. *C*, chondroma of the left fourth finger.

A large ovarian tumor of the left side was easily removed by laparotomy. There were no other intraperitoneal findings. It was a smooth mass weighing 1,900 Gm. and measuring 25 by 18.5 by 12 cm. Cut surfaces presented a grayish white, rather soft, bulging tissue arranged in lobular masses and large islands, separated by numerous but small cysts containing clear yellow limpid fluid. In some instances fibrous bands transected the cut surfaces, forming large lobulations. Some areas were deeply hemorrhagic and had yellowish foci of necrosis. Histologic sections presented a variegated surface, in which there was a pale, moist myxomatous type of stroma with large, slightly granular, lightly eosinophilic cells. These had poorly defined borders but round, finely granular nuclei. Some of the collections were associated with separation of the cell clusters into irregular small twisted columns. In other areas there was rather diffuse epithelium-like cell infiltration with little myxomatous stroma. The tumor has been studied by a number of pathologists. Five were of the opinion that it was an atypical granulosa cell tumor and not associated with endocrine function. One diagnosis was atypical arrhenoblastoma. Another was mesonephroma and another teratoma. The American Registry of Ovarian Tumors has classified the lesion as mesonephroma or teratoma. After removal of the tumor a simulated menstrual cycle was reestablished.

Two years after the removal of the ovarian tumor there was an insidious onset of weakness with loss of weight. Examination revealed a nontender, hard pelvic mass pressing on the rectum and fixing the vagina and uterus. Fever, abdominal pain, vomiting, and increase in size of the mass, with ascites, now came on rather quickly. In all 14,716 roentgens were given through various ports; but there was no response. At about the same time irregular cystic areas developed in the first, second and sixth left ribs and both shoulder girdles. The roentgenogram of the pituitary gland at this time showed an increase of the size of the sella turcica with destruction of the bony outline. A laparotomy was made on account of partial obstruction of the bowel, and the peritoneal surfaces were found to be covered with numerous irregular nodules of various sizes, microscopic examination of one of which showed peculiar myxomatous ovarian stroma resembling mucinous carcinoma. Some pathologists, however, held that it was a granulosa cell tumor, but the consensus was that it concerned a teratoid tumor. The symptoms progressively increased, and the patient died on July 8, 1946, three years after the removal of the left ovarian tumor. Unfortunately, permission to make an autopsy was not obtained.

COMMENT

It has been considered in the past that simultaneous occurrence of enchondroma and hemangioma is only coincidence. The tumors generally are not hereditary and are rarely found together, but each has been noted to occur with other defects. This case, however, presents threefold evidence of mesodermal dysplasia: multiple enchondroma, or dyschondroplasia; multiple hemangioma and a teratoid (mesodermal) tumor of the ovary. The family history does not contribute anything of significance. The history of rickets at the age of 5 years, if correct, would support Virchow's[4] contention that the development of enchondromatosis represents misplaced immature cartilaginous rests brought about by improper osseous development. The rapid development of multiple en-

4. Virchow, cited by Maffucci, A.: Movimento 3:399 and 565, 1881.

chondroma suggests a rather prominently defective mesoderm; whether the finding is an anomaly of the vessels or a neoplasm has not been determined. In structure the tumors range from the usual cavernous hemangioma to a rather cellular angioblastic tumor.

SUMMARY

The twenty-second case of dyschondroplasia associated with hemangioma (Maffucci's syndrome) is recorded. The condition is a nonhereditary type of mesodermal dysplasia. The left fourth finger was amputated because of serious deformity, and a 1,900 Gm. teratoid ovarian tumor was removed, from which intra-abdominal metastases had developed.

Laboratory Methods and Technical Notes

ARGENTAFFIN CELLS OF THE HUMAN APPENDIX

A Comparative Study of the Results Obtained with Modified Schmorl and Masson Technics

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AND
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IN THE COURSE of Lillie's current investigations of the gastrointestinal mucins, it was found that the Heidenhain-Kulschitzky cells would reduce Schmorl's[1] ferric ferricyanide lipofuscin reagent. Since the method seemed, on preliminary trial, to be about as sensitive for the detection of these cells as the commonly used Masson silver method,[2] it was decided to compare these methods on a considerable series of human appendixes. While these studies were in progress, Gomori's[3] paper appeared, in which he reported that the ferricyanides were reduced to ferrocyanides in the presence of ferric salts by the argentaffin or "enterochromaffin" cells.

In this comparative study, 464 formaldehyde-fixed appendixes cut at 5 microns were stained by the two technics, and the argentaffin cells were counted. Two appendixes had 4 cross sections per slide, 386 had 3, 67 had 2, and 9 had 1.

The technics used were:

Modified Schmorl technic:

1. Bring sections to distilled water.
2. Treat with equal parts of 1 per cent aqueous potassium ferricyanide and 1 per cent ferric chloride for 5 minutes.
3. Rinse in three changes of distilled water.
4. Stain for 1 minute in 1:5,000 new fuchsin (Color Index No. 678) in 1 per cent aqueous acetic acid.
5. Dehydrate in 95 per cent alcohol, absolute alcohol, alcohol-xylene and xylene, and mount as usual

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From the Pathology Laboratory, Experimental Biology and Medicine Institute, National Institutes of Health.

1. Schmorl, G.: Die pathologisch-histologischen Untersuchungsmethoden, ed. 15, Leipzig, F. C. W. Vogel, 1928.

2. Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948, p. 102.

3. Gomori, G.: Arch. Path. 45:48, 1948.

Results: Argentaffin granules are stained greenish blue; cell nuclei, red.

Modified Masson argentaffin technic:

1. Bring sections to distilled water.
2. Stain in ammoniacal silver nitrate in daylight for 18 to 24 hours (until sections are dark amber). This solution is made as follows: To 4 cc. of strong ammonia (28 per cent NH_4OH) add 10 per cent aqueous silver nitrate (about 40 cc.) until solution remains faintly turbid on shaking.[4]
3. Rinse quickly in distilled water.
4. Tone for 5 minutes in Burtner's[5] modified Ramóny Cajal gold toner. (The formula is 3 Gm. of ammonium thiocyanate, 3 Gm. of sodium thiosulfate and 100 cc. of 0.2 per cent gold chloride. Shake well before using.)
5. Rinse for 30 seconds in 5 per cent aqueous sodium thiosulfate.
6. Wash in running water for 2 minutes.
7. Counterstain as desired—safranin O (or others) dehydrate in acetones, acetone-xylene and xylene. Mount as usual.

Results: Argentaffin granules appear black; background, pink to brown; nuclei, red.

In comparing the two technics, adjacent cross sections were used. In such instances, often the number of argentaffin cells stained by either of the two methods exceeded considerably those shown by the other technic. A larger number of cells was demonstrated by the Masson technic in 261 cases and by the Schmorl technic in 179 cases; in 24 cases the same number of cells was demonstrated by each method. The average number of argentaffin cells per section with the modified Schmorl technic was 13.4. With the modified Masson technic the average was 14.7.

SUMMARY

Although both technics clearly differentiate the argentaffin cells, the Masson technic appears to have a slight advantage. However, it has the disadvantage of staining some extraneous material that can be mistaken for argentaffin cells. The modified Schmorl technic consistently stains the argentaffin cells greenish blue, but it shows inconsistency in the counterstaining, alternating from bright red to faint pink.

Hence, the two methods appear to have nearly equal value, and the Schmorl would be perferred when the relatively brief time required is an important consideration.

4. Lillie,[2] p. 187.
5. Burtner, H. J.: Unpublished data.

Books Received

HETERO-SPECIFIC ALTERATION THERAPY: A NEW TREATMENT FOR PULMONARY TUBERCULOSIS BASED ON SPECIFIC CELLULAR ALTERATION PRODUCED BY A MIXED AUTOLYSATE OF TYPHOID BACILLI AND GONOCOCCI. By Susumo Nukada, M.D., Ph.D., and Chieko Ryu, M.D., of the Nukada Institute and Sanatorium, Inage, Chiba-City, near Tokyo. Paper. Pp. 80, with 5 illustrations. Tokyo, Kyoto. The Japan Medical Publications Co., Ltd., 1948.

In 1924, Nukada and Matsuzaki reported that after rabbits had been vaccinated with isologous and various kinds of heterologous bacteria the antitoxic resistance of the heart showed remarkable variation even ten days after the last inoculation and that in animals vaccinated with a certain kind of heterobacteria the resistance of the heart was greater than that of nonvaccinated control animals. Subsequently, Nukada and his collaborators studied the fluctuations in the resistance or defensive power against bacterial infection of animals following vaccination (immunization) with different varieties of heterobacteria to note which produced the best protection. They found that a mixture of typhoid bacilli and gonococci produced the best "anti-tubercular" resistance. In the years from 1931 to 1937 Nukada and Ryu determined the minimal doses required to affect the course of experimental tuberculosis in rabbits and guinea pigs given large amounts of virulent human tubercle bacilli intravenously. Most of the treated animals survived longer than controls or those treated with colon bacillus or pneumococcus vaccine. Later an attempt was made to isolate the active principle, and it was found that "anti-tubercular" resistance was produced in guinea pigs by a mixture of an "autolysate" (autodigestion product) of typhoid bacilli and gonococci, to which they give the name "heterosate," and that this proved greater than the resistance produced with the bacilli themselves. The authors feel that the results produced with "heterosate" are due to an increase of resistance or defensive power against the tubercle bacilli developed as a "specific alteration" of the tissue cells. Tests were made on tuberculous patients (weekly subcutaneous injections), the doses being gradually increased, to avoid irritation, and improvement similar to that noted in animals resulted, particularly as pertained to reduction of temperature. Satisfactory results were noted in pulmonary tuberculosis.

The book is printed in English and presents tabulations of numerous experiments submitted in evidence. Although the data might be accepted as observational, it might be questioned whether the conclusions drawn warrant clinical therapeutic application since subjective findings may be so misleading. Scientifically, there are wide gaps in the reasoning applied to justify therapeutic conclusions. The "heterosate" prepared from heat-killed organisms (53 C. for one hour) and containing phenol can hardly be called an autolysate in the strict definition of this phenomenon. There is also some confusion as to its effectiveness as a preventive or a therapeutic agent. Preventive tests proved a lesser incidence of tuberculosis in nurses (44 persons), and therapeutic tests made in 932 cases of pulmonary tuberculosis gave "very satisfactory" results. The injection of "heterosate" never produced secondary ill effects. It is proposed to call this method of treatment of pulmonary tuberculosis "hetero-specific alteration therapy" or "heterosate therapy," terms based on the conception that a cellular

alteration is produced by subcutaneous injections of a mixed autolysate (term questioned by the reviewer) of typhoid bacilli and gonococci ("heterosate"), whereby the resisting or defensive power of the tissue cells is increased specifically against tubercle bacilli and, in consequence of this, the cure of the disease promoted. The book should be read and evaluated critically by those interested.

CANCRO: CARCINOGENESE, CARCINORESISTENCIA, CARCINOINIBICAO. By Michel Mosinger, Arquivos de anatomia patólogica, patologia correlativa e neuroergonologia, volume 33—1946-1947 (Tome 1). Pp. 287. Coimbra, Portugal: Coimbra Editora, Limitada, 1947.

This entire number is written (in French and Portuguese) by Prof. Dr. Michel Mosinger, director of the Institute of Pathologic Anatomy of the University of Coimbra. It contains summaries and conclusions of experiments performed since 1936 by the author and his associates, together with brief bibliographies, references to the original publications, and 252 figures, mostly photomicrographs. The experimental work includes, among others, studies of guinea pigs, rats and mice treated with estrogens and carcinogens.

CORRECTION

In the article by Dr. George Gomori, "The Chemical Character of the Enterochromaffin Cells," in the January issue (Arch. Path. 45:48, 1948), in the last paragraph on page 49, "strong solution of iodine U.S.P." was substituted for "Lugol's solution," which as understood by histologists is the Gram modification, containing 0.33 per cent iodine, while strong solution of iodine U.S.P. contains 5 per cent iodine.